ALUMINUM COMPOUNDS VOL III #43
COPIES OF ARTICLES CITED BUT
NOT USED IN MONOGRAPH SUMMARY

VOLUME 3

GRAS MONOGRAPH SERIES

ALUMINUM COMPOUNDS

(COPIES OF ARTICLES CITED BUT NOT USED IN MONOGRAGH SUMMARY)

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

JUNE 8, 1973

prepared by

Tracor Jitco, Inc.

HYPERALUMINÆMIA FROM ALUMINIUM RESINS

SIR,—Professor Wrong and Dr. Swales (Nov. 28, p. 1130) seem to have missed the point of our article.[1] Aluminium resins cause hyperaluminæmia in 30% of renal-failure patients. I do not know whether hyperaluminæmia is harmful, and, as the article said, it requires further work to determine the toxic effects of hyperaluminæmia. I am concerned about the use of aluminium hydroxide to lower serum-inorganic-phosphate levels, but I am not aware that an efficient trouble-free alternative to aluminium hydroxide has been found. The argument that aluminium must be safe because it is the third most abundant element in nature is a non-sequitur. Silicon is an abundant element in nature, but silicosis is a serious disease. The toxicity of hyperaluminæmia is a possibility, and if one could easily measure the level of serum-aluminium and exclude hyperaluminæmia I would agree with Professor Wrong and use the mixture of calcium and aluminium resins he advocates. Unfortunately, it is very difficult to measure aluminium specifically, and until it can be measured easily aluminium resin should be regarded with some suspicion, and its use limited to centres where serum-aluminium levels can be regularly monitored.

Faculty of Life Sciences,
University of the Negev,
Israel

G. M. BERLYNE.

Lancet

No. 7685 vol II for 1970
p. 1253

Vol 1 for 1972
No. 7

Maudsley Hospital,
Denmark Hill,
London S.E.5.

RACHEL ROSSER.

ALUMINIUM TOXICITY

SIR,—Dr. Thurston and his colleagues (April 22, p. 881) make some interesting points about aluminium toxicity in rats, and offer a number of speculations about our work on this subject (March 11, p. 564). We believe that your readers should consider the following points:

(1) They suggest that periorbital bleeding in our rats was not due to aluminium intoxication but rather was a non-specific finding due to "sickness and uræmia". This statement is incorrect and is based on their experience in Manchester with a colony of rats of unstated breed in which there were "other animals in a poor general state". It is a prior condition of all toxicity experiments that the colony should be healthy. In Beer-Sheba our rat colony is healthy, lives in a rigidly controlled environment, and is looked after by one of us (R. Y.) who is a qualified veterinary surgeon trained in Holland. There are more than 1000 rats per day in the colony. In the past three years there has not been a single case of periorbital bleeding in rats in the colony other than in rats receiving aluminium salts.

(2) Periorbital bleeding occurs in rats with higher plasma levels than are obtained in rats on oral aluminium hydroxide. We published a table of plasma-aluminium levels and a table of frequency of periorbital bleeding showing the relationship between mode of administration, salt of aluminium, and periorbital bleeding. We are not surprised that Dr. Thurston and his co-workers did not get symptoms of aluminium intoxication with oral $Al(OH)_3$. Neither did we. The absence of plasma-aluminium levels in their paper is regrettable, because it prevents comparison of results.

(3) They were unfortunate to give the aluminium hydroxide as a mix with dry meal, because rats are not noted for their fastidious eating habits. They scatter food on the floor of their

1. Wilson, W. A. *Med. Care, Lond.* 1970, **8**, 98.
2. Lee, J. A. H., Morrison, S. L., Morris, J. N. *Lancet*, 1957, ii, 785.
3. Lee, J. A. H., Morrison, S. L., Morris, J. N. *ibid.* 1960, i, 170.
4. Ashley, J. S. A., Howlett, A., Morris, J. N. *ibid.* 1971, ii, 1358.

cages, as can be seen by using metabolic cages. This is reduced by using pellet foods, but gavage is more certain. Because of this, the amount of aluminium actually consumed by the rats of Dr. Thurston and his colleagues is in the realm of guesswork.

(4) Periorbital bleeding is the most reliable sign of aluminium intoxication in the rat when plasma levels approach those seen in patients with advanced renal failure and receiving 6–8 g. of $Al(OH)_3$ a day (apparently 3 times the dose that is now used by Dr. Thurston and his associates).

For these reasons we advise that the use of aluminium salts in acute renal failure be regarded with caution and scientific detachment.

G. M. BERLYNE
R. YAGIL
J. BEN-ARI
G. M. DANOVITCH.

Department of Nephrology,
Negev Central Hospital,
Beer-Sheba, Israel.

Analyst 73: 501-2 (1948)

Thioglycollic Acid as an Inhibitor for Iron in the Colorimetric Determination of Aluminium by means of "Aluminon"

By E. M. CHENERY

THE determination of small quantities of aluminium is rarely free from interference by iron, as these two elements almost invariably occur together. Several methods have been devised to overcome iron interference but none is entirely satisfactory. Time-consuming methods in which iron is removed[1] or compensated for[2,3] are usually employed.

Thioglycollic acid was first used as an inhibiting agent for iron by Hutchinson and Wollack[3] in the gravimetric estimation of aluminium by means of 8-hydroxyquinoline. The aluminium hydroxyquinolinate was precipitated in presence of the reddish-purple ferrous complex. With the object of improving a compensation method[2] the present writer investigated the use of thioglycollic acid as an iron inhibitor in acid solutions. Fixation of iron as a colourless complex took place under the original conditions of aluminium-aluminon lake development, *viz.*, buffering at *p*H 4·0 and heating for 4 minutes at 100° C. In the procedure recorded below, 0 to 10 μg. of aluminium (in 5 ml.) can be accurately determined in presence of 200 μg. of iron, more than 2000 μg. of magnesium and 200 μg. of phosphate ion. Chromium and rare earths should be removed when present in amounts more than one-eighth that of the aluminium; but as this is very unlikely in water, biological material and clay minerals, these elements may usually be ignored. No inhibitor is required when the Al : Fe ratio is 15 : 1 or more. The effect of iron on aluminium determinations by this method is shown in the following table.

Fe added, μg.	0	50	100	200	500
Al found, μg.	4·75	4·75	4·8	5·0	5·5
Difference, %	0	0	1·0	5·3	15·8

REAGENTS—

Aluminon reagent—Ammonium aurine tricarboxylate, 0·75 g.; gum acacia, 15 g.; ammonium acetate, 250 g.; concentrated hydrochloric acid (A.R.), 189 ml.; dissolved separately, mixed, filtered and made up to 1500 ml.

Thioglycollic acid—One ml. diluted to 100 ml.

Aluminium sulphate standard—Stock solution containing 250 p.p.m. of aluminium and containing 4 ml. of concentrated nitric acid per litre. Ten ml. of this diluted to 250 ml. gave the 10-μg. aluminium standard, aliquots of which were used in preparing the curves. The stock solution must be analysed gravimetrically for aluminium, especially in the tropics, where loss of water of crystallisation may lead to errors.

PROCEDURE—

Since the working range of the red aluminon lakes is from 0 to 10 μg. in 5 ml., the test solutions should be diluted to this after preliminary rough trials, taking care they are not more acid than the 10 μg. aluminium standard. The test solutions containing not more than a total of 10 μg. of aluminium in 3 ml. are pipetted into Pyrex test tubes (15 × 150 mm.) graduated at 5 ml. Five drops or 0·2 ml. of the dilute thioglycollic acid are added and the solutions well mixed. One ml. of aluminon reagent is then added and the contents of the tubes are made up to 5 ml. and mixed by agitation. The solutions are then heated for exactly 4 minutes in a strongly boiling water-bath and allowed to cool slowly. After 1½ to 2 hours or longer the levels are made up to the mark again and the colours measured in a colorimeter. Any type of colorimeter can be used; good results have been obtained with wedge and dipping instruments and also with the Lovibond tintometer. Most of the work done on this method was performed with a photo-electric instrument (Cenco Photelometer). For more precise determinations, small measuring flasks of 10- to 25-ml. capacity are substituted for the graduated test tubes. Batches of 24 test solutions can be dealt with very conveniently at one time. All the reagents were quite stable over a period of 6 months and the lakes, after standing for 1½ hours, remained unchanged for a further 24 hours.

References

1. Strafford, N., and Wyatt, P. F., Analyst, 1943, 68, 319.
2. Chenery, E. M., Ph.D. Thesis, University, Lond., 1936.
3. Hutchinson, G. E., and Wollack, A., *Connecticut Acad. Trans.*, 1943, **35**, 73.

Department of Agriculture
Port of Spain
Trinidad, B.W.I.

January, 1944

Indust. Engin. Chem: 24: 403-405 (1932)

Occurrence and Determination of Aluminum in Foods

I. Determination of Aluminum in Organic Materials

GERALD J. COX, E. W. SCHWARTZE, RAYMOND M. HANN, AND RICHARD B. UNANGST, Mellon Institute of Industrial Research, Pittsburgh, Pa., AND J. L. NEAL, Aluminum Co. of America, New Kensington, Pa.

Aluminum in organic tissues is determined colorimetrically as a lake with aurin tricarboxylic acid. Exact details of ashing procedure and conditions for lake formation are given. Simplification of technic and increased precision over previously described methods are attained by use of a permanent color standard and a combined reagent for lake development. Mercaptoacetic (thioglycolic) acid is used as a test for iron.

DURING the course of an investigation of the suitability of various metals for the construction of cooking utensils and food containers, it has been necessary for the authors to determine very small amounts of the respective metals that have been taken up by foodstuffs from the surfaces with which they have been in contact. These analytical procedures have been applied both to the foodstuffs as prepared for the table and to the tissues of animals that have received known concentrations of metallic salts in their daily diet. This paper describes the procedure that has been followed for the determination of aluminum. The concentrations of aluminum thus determined have been as small as 0.1 p. p. m.

A colorimetric method for aluminum, based on the procedures of Myers, Mull, and Morrison (*2*), and of Winter and his associates (*7, 8, 9*), has been used. The method, in brief, consists of ashing the material at as low a temperature as possible, solution of the ash, precipitation of aluminum as the phosphate adsorbed on an excess of ferric phosphate, removal of iron, development of a color by means of the aurin tricarboxylic acid lake, and determination of the aluminum by comparison with a fixed color standard.

PROCEDURE

Fresh washed tissue (5 to 150 grams) is dried overnight in a platinum dish at 110° C. It is then placed on a nichrome-triangle in a cold electrically heated muffle, and the temperature is gradually raised during the day to a faint red heat. The ashing process is completed overnight while a slow current of oxygen is passed into the muffle to hasten the combustion.

The ash is treated with 10 cc. of concentrated hydrochloric acid and 25 cc. of water. The resulting solution is evaporated to dryness to dehydrate silica. To the residue are added 10 cc. of 3 *M* hydrochloric acid and 25 cc. of water, and the solution is boiled for 5 to 10 minutes. The solution is centrifuged at 1800 r. p. m. for 5 minutes and decanted into a 100-cc. Erlenmeyer flask. The residue, if any, is washed and discarded; but if carbon remains in the residue, the latter is transferred to a platinum crucible and dried. The silica is volatilized with hydrofluoric acid and sulfuric acid, followed by gentle ignition. The residue is then fused in a small amount of equal parts of sodium and potassium carbonates and dissolved in the main solution.

To the solution of the ash are added 1 cc. of concentrated nitric acid (specific gravity, 1.42) and 1 cc. of 0.1 *M* ferric sulfate. The solution is evaporated to about 10 cc. and diluted with water to approximately 60 cc. Five cc. of 1 *M* monosodium phosphate and 2 cc. of 0.04 per cent bromophenol blue are added and then 7 *M* ammonium hydroxide until a permanent precipitate is formed. Next the pH is brought to 4.2 by the addition of 3 *M* sodium acetate. The mixture is centrifuged for 5 minutes at 1800 r. p. m., and the liquor is discarded.

The precipitate of ferric and aluminum phosphate is dissolved in 0.5 cc. of 6 *M* hydrochloric acid and 1.25 cc. of glacial acetic acid, and approximately 15 cc. of hot water are added. Five cc. of 6 *M* sodium hydroxide are stirred in with a glass rod, and the mixture allowed to stand for an hour with frequent stirring. The glass rod is washed, and the ferric hydroxide thrown down by centrifuging for 5 minutes at 1800 r. p. m. The solution is decanted through two 9-cm. filter papers prepared by washing with warm 1.2 *M* sodium hydroxide, followed by hot water until practically all alkali is removed. The filtration is made into a 100-cc. volumetric flask containing 1 cc. of 6 *M* hydrochloric acid. The precipitate remaining in the centrifuge tube is washed once by stirring with 20 cc. of hot water and centrifuging. The washings are decanted through the filter. The ferric hydroxide is not transferred to the paper. The filter paper is thoroughly washed with water, the solution cooled, made faintly acid to litmus, and diluted to 100 cc.

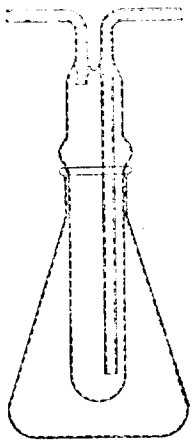


FIGURE 1. FLASK AND CONDENSER FOR LAKE DEVELOPMENT

Twenty cubic centimeters of the solution are measured into a dry 250-cc. glass-stoppered Erlenmeyer flask. Then there are added 25 cc. of a solution containing, in 1 liter, 1 mole of ammonium acetate, 1 mole of ammonium chloride, 80 cc. of 0.1 per cent ammonium aurin tricarboxylate (aluminon), and 60 cc. of 6 *M* hydrochloric acid. The pH at this point must be between 4.5 and 5.5. A snugly fitting test-tube condenser (Figure 1) is placed in the neck of the flask, and the solution is boiled for 1 minute, timed from the first appearance of steam bubbles. The solution is cooled for 1 minute under the condenser and then to room temperature in running water.

Enough 1.6 *M* ammonium carbonate (4.8 to 5 cc.) is added with gentle shaking to give a final pH of 7.1. The flask is stoppered and shaken up and down twenty times. Carbon dioxide is released by cautiously removing the stopper, and

the flask is allowed to stand for 20 minutes, with the stopper loosened, for the excess dye to be decolorized. The pH should now be within the limits 7.0 and 7.3, but preferably at 7.1.

The concentration of the aluminum is determined immediately in a Dubosq colorimeter by comparison of the intensity of the lake color with a standard containing 5 cc. of 0.04 per cent thymol blue and 8 cc. of 6 *M* hydrochloric acid in 500 cc. of solution. The aluminum lake solution is set at a constant depth of 30 mm., and the depth of the standard is varied for the color matching.

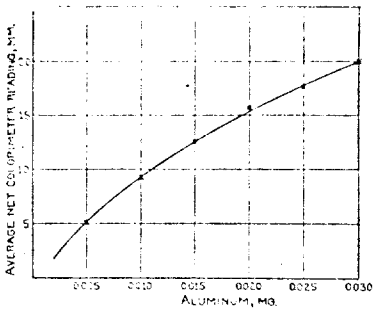


FIGURE 2. NET COLORIMETER READINGS OBTAINED WITH ADDED ALUMINUM

The amount of aluminum present in the aliquot is determined by reference to a curve plotted from the colorimeter readings obtained when varying amounts of aluminum are subjected to the entire scheme of analysis beginning with the ash (Figure 2).

DISCUSSION OF TECHNIC AND REAGENTS

In the initial solution of the ash of heavy carcasses it is necessary to use 25 cc. of concentrated hydrochloric acid and 25 cc. of water and to aid the process by trituration of bone ash with a flattened glass rod. The second solution is effected by 20 cc. of concentrated hydrochloric acid and 150 cc. of water. An aliquot, usually one-fifth, is taken for the rest of the procedure. No addition of monosodium phosphate is necessary in the analysis of carcasses.

The reaction of pH 4.2 should be very closely controlled in the precipitation of the phosphates of iron and aluminum, to effect a quantitative removal of aluminum and insure its freedom from calcium phosphate.

Carl Schleicher and Schüll No. 589 white ribbon filter paper was used in preparing the iron-free filtrate from the ferric hydroxide precipitation. These filtrates must be entirely iron-free, and the mercaptoacetic acid test has been found most satisfactory. When 10 cc. of the solution are treated with a drop of mercaptoacetic acid (*1*) and then made alkaline with ammonium hydroxide, there should be no pink color developed. The writers have found that iron is not completely removed by centrifuging. Filtration through one paper removes part of the residual iron; two papers remove all of it. The process seems to be one of adsorption rather than of filtration.

The reagent used for development of the aluminum lake is based on the separate solutions used by Winter, Thrun, and Bird (*9*).

The combined reagent obviously reduces the manipulations and insures more uniform conditions during the lake development.

Because of variations in the escape of carbon dioxide in the decolorization of the excess dye after lake development, the given procedure for shaking must be rigidly adhered to in order to insure a final pH of 7.1. If the solution is not shaken enough, the pH will be too low for complete change of the dye to the yellow form; if the shaking is too vigorous or prolonged, the pH will be high enough to result in appreciable fading of the lake color.

The thymol blue permanent standard used by Thrun (*5*) in the study of the stability of aluminum lakes is very convenient and preferable to standards prepared from aluminum solutions. The pink color is stable over periods of at least 6 months. The color match is not perfect, but readings are readily duplicated to give thoroughly reliable analyses. The precision of the readings is such that the probable error of sixteen random observations at about 30 mm. is ± 0.10 mm. A random selection of some early readings, made by another trained observer, of aluminum lake against aluminum lake shows the probable error of ± 0.25.

All reagents were made up from large stocks of chemicals of the same lot number in order to maintain a uniform blank in all analyses. Mallinckrodt's C. P. quality sodium hydroxide crystals ($NaOH + H_2O$) have been found to be practically aluminum-free. Its use obviates the tedious preparation of the alkali solution from aluminum-free sodium. The 6 *M* solution was prepared by direct weighing without subsequent standardization. The 6 *M* hydrochloric acid was prepared by distilling an approximately 6 *M* solution and collecting the last three-fourths of the distillate. The aluminon of the Fales Chemical Company was used.

The empirical curve employed is an inverse curve compared with that of Winter, Thrun, and Bird (*9*), as the depth of the standard in the colorimeter was varied rather than that of the unknown. The curve automatically corrects for the blank of the reagents, as it is a record of the colorimeter readings obtained when definitely known amounts of aluminum enter the analytical system. The curve shown in Figure 2 represents the net colorimeter readings obtained in a series of observations of the intensity of color produced by known amounts of pure aluminum salts added at the beginning of the procedure. The reagents used produced color equivalent to 12.5 to 14 mm. of the thymol blue standard. In routine analyses a blank control was invariably carried through to secure the net reading of the colorimeter, a reading which is obviously altered by the size of the aliquot required. The curve shown in Figure 2 is not reproduced for use, as its form and position depend on the quality of all reagents. It is necessary to prepare a curve for each set of reagents, a process that is simply a matter of "blanking" the reagents.

In a system which involves the use of standards prepared from known amounts of aluminum, the reagents involved in the preparation of those standards always introduce an additional unknown amount of aluminum. An inherent property of an empirical curve based on the use of a permanent standard and the values of aluminum entering the system is that it corrects for the entire blank of the procedure.

At the beginning of this investigation two general methods were available—the chemical and the spectrographic procedures. The latter presented the advantage of speed and the complete absence of contamination of the ash by the addition of reagents, but at that time was only roughly quantitative and required the installation and maintenance of expensive equipment. The advances made by Myers, Mull, and Morrison (*2*), and, during the period of the present studies, by Winter and associates (*5*, *7*, *8*, *9*) and by Underhill and Peterman (*6*), have influenced the choice of the chemical method here used.

After an exhaustive investigation of ashing by the wet method—i. e., by sulfuric acid digestion with the addition of perchloric acid or nitric acid—and of the alternative ashing by ignition, the latter was chosen. In the wet-ashing procedure, some aluminum was introduced by the reagents, and containers were etched to give insoluble residues which were difficult to handle. These objectional features were absent

from the dry-ashing procedure. The authors were not able to demonstrate a loss of added aluminum in smoke evolved in ignition of various materials.

An investigation was made of the removal of iron, which interferes in the colorimetry, by means of cupferron. The removal effected was very complete, but the method was cumbersome and required an ashing procedure to remove the excess of cupferron. Iron is not removed completely by centrifuging, but, by adsorptive filtration through two filter papers, the mercaptoacetic acid test becomes uniformly negative.

A study of the interference of silica in colorimetry reveals that it is sufficiently removed by dehydration so that hydrofluoric acid treatment is unnecessary.

The absolute quantity of aluminum present in a given tissue, of course, cannot be determined by any method. Recourse must therefore be had to experiments on the recovery of added aluminum to prove the accuracy of a method. A well-mixed sample of ground beef liver showed, after the manipulations of grinding and mixing, 5.0 p.p.m. of aluminum. When 0.05 mg. of aluminum was added to 25 grams of the beef liver (yielding 0.125 mg. of aluminum), making a total apparent aluminum content of 0.175 mg. before ashing, 0.1785 and 0.1725 mg. were recovered. When the aluminum was added to the ash, 0.1725 and 0.1715 mg. were recovered.

The reader is referred to the articles cited (*2, 5, 6, 9*) for other details of technic and for the underlying principles of the method. Other articles in this series (*3, 4*) have been published, and further contributions will follow. Copies may be obtained by addressing the senior author.

Acknowledgment

In the development of this method W. H. Bradley, Mary L. Dodds, A. D. Mclavain, F. J. Murphy, and Helen B. Wigman have rendered technical assistance. The authors also wish to acknowledge their indebtedness to G. D. Beal, R. W. Bridges, and F. C. Frary for many helpful suggestions.

Literature Cited

(1) Lyons, E., *J. Am. Chem. Soc.*, **49**, 1916 (1927).
(2) Myers, V. C., Mull, J. W., and Morrison, D. B., *J. Biol. Chem.*, **78**, 595 (1928).
(3) Schwartze, E. W., Murphy, F. J., and Cox, G. J., *J. Nutrition*, **4**, 211 (1931).
(4) Schwartze, E. W., Murphy, F. J., and Hann, R. M., *Ibid.*, **2**, 325 (1930).
(5) Thrun, W. E., *J. Phys. Chem.*, **33**, 977 (1929).
(6) Underhill, F. P., and Peterman, F. J., *Am. J. Physiol.*, **90**, 1 (1929).
(7) Winter, O. B., *J. Assoc. Official Agr. Chem.*, **13**, 220 (1930).
(8) Winter, O. B., and Bird, O. D., *J. Am. Chem. Soc.*, **51**, 2964 (1929).
(9) Winter, O. B., Thrun, W. E., and Bird, O. D., *Ibid.*, 2721 (1929).

Received January 18, 1932. Present addresses of authors are as follows: E. W. Schwartze, Medical Department, University of Georgia, Augusta, Ga.; R. M. Hann, National Institute of Health, Washington, D. C.; R. B. Unangst, Aluminum Co. of America, New Kensington, Pa.; J. L. Neal, 1125 W. Onondaga St., Syracuse, N. Y.

• • • • •

II. Aluminum Content of Foodstuffs Cooked in Glass and in Aluminum

George D. Beal, Richard B. Unangst, Helen B. Wigman, and Gerald J. Cox

Mellon Institute of Industrial Research, Pittsburgh, Pa.

Foods cooked in glass and in aluminum have been analyzed for aluminum, and the average increase has been calculated. The taking up of aluminum by neutral foods is negligible: acid and alkaline foods are relatively more corrosive. In no case, however, is sufficient aluminum dissolved from utensils to interfere seriously with phosphorus absorption. An average daily intake of aluminum in case all foods are cooked in aluminum is estimated at 12 mg., of which about 5 mg. is derived from the utensils.

A NECESSARY qualification of all materials that come into contact with food during its production, distribution, cooking, or service is that they must not in any way depreciate the quality of the food. Primarily, it is not permissible that any essential constituent of the food be removed or any harmful substance added. Of secondary importance is the requirement that a specific undesirable taste or odor should not be imparted and that a discoloration should not be produced in foods by containers during any of the operations mentioned. It is, of course, also of importance that the utensil be resistant to corrosion by foods in order that it survive use in service.

Aluminum is today the most popular of all materials for the construction of cooking utensils because of its light weight, attractive appearance, durability, high heat conductivity, and ease of cleaning and maintenance for constant use. This wide popularity of aluminum after about thirty years of employment is convincing evidence that it is satisfactorily resistant to food attack, and that no harm results from eating foods customarily prepared in aluminum vessels. It has been shown elsewhere (*14*) that only barely detectable amounts of aluminum appear in the tissues, following diets containing large amounts of aluminum; the results to be expected (phosphorus starvation) when overwhelming doses of soluble aluminum salts are fed have also been described (*3*). In the present paper a study of the amounts of aluminum which enter foods by contact with aluminum in a variety of culinary practices will be recorded. In every case the amount of aluminum added to foods by utensils made of it has been found to be small and far below that necessary to produce phosphorus starvation and its sequelae, the only abnormal conditions which the authors were able to cause by administering to experimental animals excessively large doses of aluminum.

Previous Studies of Aluminum Corrosion

A few quantitative and many qualitative studies of the corrosion of aluminum by foods have been made, especially in European laboratories. The latter have consisted of observations of pitting, polishing, or staining of aluminum surfaces exposed to foods; of precipitates in water and clear liquids; of discoloration of foods; and of alleged changes in taste. Occasionally, chemical demonstrations of the presence of aluminum in the food have been mentioned. Most of these reported findings, both qualitative and quantitative, are of little value now, as they were obtained by the use of articles constructed of various grades of early commercial aluminum

The Effect of Aluminum Hydroxide Upon Food Utilization in Human Subjects*

By

RAYMOND D. GRONDAHL, M.D.

AND

EDWARD S. WEST, Ph.D.
PORTLAND, OREGON

IT IS well known that aluminum hydroxide is an excellent protein and enzyme adsorbent. In recent years it has also been demonstrated that aluminum hydroxide markedly inhibits gastric digestion. Komarov and Krueger (1) have shown that feeding aluminum hydroxide to dogs inhibits the secretion of gastric juice and also decreases the activity of pepsin. This was observed even in gastric pouches not in direct contact with the agent. Komarov and Komarov (2) have pointed out that aluminum hydroxide, when added to the gastric juice of dogs in vitro, quantitatively removes the pepsin from solution. Schiffrin and Komarov (3) have shown that the presence of aluminum ions in a solution of the enzyme inhibits proteolytic activity at pH values at which the enzyme is not precipitated. In view of the above facts it seemed of some interest and importance to determine the effect of prolonged administration of aluminum hydroxide to human subjects on the digestion and absorption of foods, since such treatment is common practice in the handling of ulcer cases.

Beazell, Schmidt, and Ivy (4) found that aluminum hydroxide added to pancreatin in vitro did not alter the tryptic or lipolytic activity of the preparation. These workers also demonstrated that prolonged administration of aluminum hydroxide to dogs on a standard diet did not alter the fecal fat or nitrogen content. So far as we have been able to determine, the present work represents the first report dealing with the effect of aluminum hydroxide on digestion and absorption in human subjects.

EXPERIMENTAL

A healthy subject with no demonstrable gastro intestinal pathology was selected to serve as a normal control. The subject first collected twenty-four hour urine and feces samples for seven days without previous administration of aluminum hydroxide. This was followed by a period in which 60 cc of amphogel was taken in six divided doses spaced throughout the day. Ordinarily, doses were taken after each meal, midway between meals, and before retiring. A glass of water was taken with each dose. A normal average diet was ingested during both periods.

A hospital patient with a known gastric ulcer was chosen as subject for studying the effect of aluminum hydroxide under therapeutic conditions. The gastric lesion in this subject was shown by x-ray and gastroscopy to be located on the lesser curvature about nine centimeters from the pylorus. This subject was put on ulcer management until evidence of healing of the lesion was shown by gastroscopy. At that time a diet of C-180, P-75, F-80, given at 3 feedings, and supplemented by 3 ounces of milk given at hourly intervals from 7:30 A.M. to 7:30 P.M., was started. Vitamin supplements were supplied. This regimen was continued for twelve days to serve as control period, and then 5 cc of amphogel, given hourly from 8:00 A.M. to 8:00 P.M., was added to the control diet. Twenty-four hour urine and feces samples were collected for analysis.

TABLE I

Composition of feces and urine for 24 hour periods

NORMAL SUBJECT

No Aluminum Hydroxide in Diet

Fecal Fat Grams per 24 hours	Fecal Carbohydrate* Grams per 24 hours	Urine Nitrogen Grams per 24 hours	Fecal Nitrogen Grams per 24 hours	U.N./F.N.
6.00	0.31	14.9	2.24	6.7
4.90	0.46	14.5	1.90	7.6
		14.9	2.70	5.5
Av. 5.45	Av. 0.39	19.2	2.34	8.3
		18.5	2.86	6.5
		17.1	2.24	7.6
		17.1	3.10	5.5
		Av. 16.6	Av. 2.49	Av. 6.6

Aluminum Hydroxide Present in Diet

Fecal Fat Grams per 24 hours	Fecal Carbohydrate* Grams per 24 hours	Urine Nitrogen Grams per 24 hours	Fecal Nitrogen Grams per 24 hours	U.N./F.N.
7.00	0.11	16.3	2.95	5.5
8.24	0.56	13.7	2.38	6.0
9.88	0.20	13.0	2.08	6.3
		15.6	2.02	7.7
Av. 8.36	Av. 0.29	12.2	1.93	6.3
		16.9	2.00	8.5
		Av. 14.6	Av. 2.23	Av. 6.7

*Carbohydrate as glucose.

Urine samples were collected in bottles containing toluol as preservative. Feces were collected, placed in one half gallon jars, 1 volume of feces mixed with 3 volumes of water and a sample of 2-3 cc removed for pH determination. 60 cc of concentrated sulfuric acid was stirred into the mixture and after several days, following emulsification, the acidified mixture was diluted to 1000 cc and reserved for analysis. pH determinations on the urine and feces were made with the glass electrode using a Beckman pH meter. Titratable acidity of urine was estimated by titration with .1 N

*From the Department of Biochemistry, University of Oregon Medical School, Portland, Oregon.
Submitted Sept. 5, 1944.

sodium hydroxide in the presence of potassium oxalate, using phenolphthalein as an indicator. Nitrogen determinations were done according to the Kjeldahl method. The determination of total carbohydrate in the feces involved hydrolysis with 0.5 N sulfuric acid for 5 hours, precipitation according to the iron method of Steiner, Urban, and West (5), and estimation of fermentable sugar in the filtrates according to the method of Somogyi (6). Fat content of the feces was estimated according to the method of Saxon (7).

TABLE II

Composition of feces and urine for 24 hour periods

GASTRIC ULCER PATIENT

No Aluminum Hydroxide in Diet

Fecal Fat Grams per 24 hours	Fecal Carbohydrate Grams per 24 hours	Urine Nitrogen Grams per 24 hours	Fecal Nitrogen Grams per 24 hours	U.N./F.N.
0.64	0.52	10.2	0.15	65.0
0.34	0.24	10.0	0.44	22.5
0.34	0.28	10.5	0.36	28.7
		10.3	0.52	19.8
Av. 0.44	Av. 0.35			
		Av. 10.3	Av. 0.37	Av. 34.0

Aluminum Hydroxide Present in Diet

Fecal Fat Grams per 24 hours	Fecal Carbohydrate Grams per 24 hours	Urine Nitrogen Grams per 24 hours	Fecal Nitrogen Grams per 24 hours	U.N./F.N.
0.64	0.41	7.7	0.80	10.0
0.70	0.14	7.4	1.43	5.2
	0.38	6.1	0.20	29.0
Av. 0.67		14.0	2.56	5.5
	Av. 0.31	12.0	0.52	24.0
		Av. 9.50	Av. 1.10	Av. 14.7

TABLE III

Urine Titratable Acidity and Urine and Feces pH for 24 Hour Periods

NORMAL SUBJECT

No Aluminum Hydroxide in Diet

Urine pH	Titratable Acidity cc.0.1N	Feces pH
6.42	205.7	6.15
5.45	479.3	5.90
5.40	470.1	6.10
5.25	505.0	5.90
5.48	456.0	5.90
5.30	545.6	6.00
5.32	459.4	6.00
Av. 5.51	Av. 445.8	Av. 5.99

Aluminum Hydroxide Present in Diet

Urine pH	Titratable Acidity cc.0.1N	Feces pH
5.80	377.2	6.95
5.95	191.9	7.15
6.28	91.0	6.90
5.72	121.5	5.98
5.30	229.3	6.72
5.70	201.0	6.50
5.65	200.0	6.92
5.15	414.2	6.80
5.35	360.4	6.65
5.50	262.8	6.70
6.30	224.4	6.90
5.70	212.0	6.40
6.30	189.0	6.90
5.60	307.0	6.30
Av. 5.72	Av. 241.5	Av. 6.69

DISCUSSION

In the normal subject, the collection of samples was very carefully done and as can be seen from the tables, remarkably consistent results were obtained. In order to show any change in protein utilization the ratio of nitrogen excreted in the urine to the nitrogen excreted in the feces was calculated. The average values for this ratio were 6.6 and 6.7 in the control and aluminum hydroxide periods respectively, which indicates no interference of aluminum hydroxide with protein utilization in this case.

Accurate daily feces sampling in the study of the hospitalized subject could not be secured because of severe constipation and feces impaction. Nitrogen ratios during the control period averaged 34.0, while the average was 14.7 with aluminum hydroxide. Further study to confirm this was not done because cooperation in collecting samples could not be obtained.

Fat and carbohydrate determinations on the feces of the normal subject were carried out on the last two days of the control period and on the last three days of the aluminum hydroxide period. The results show that the ingestion of aluminum hydroxide was without appreciable effect upon the utilization of fat and carbohydrate. Similar results were obtained in case of the gastric ulcer patient, who, however, excreted much less

TABLE IV

Urine Titratable Acidity and Urine and Feces pH for 24 Hour Periods

GASTRIC ULCER PATIENT

No Aluminum Hydroxide in Diet

Urine pH	Titratable Acidity cc.0.1N	Feces pH
5.75	283.8	7.95
6.26	177.5	8.15
5.85	334.3	8.42
5.55	332.9	8.05
6.30	199.5	8.30
6.35	199.8	8.15
5.15	320.0	7.40
Av. 5.88	Av. 263.9	Av. 8.06

Aluminum Hydroxide Present in Diet

Urine pH	Titratable Acidity cc.0.1N	Feces pH
5.72	196.9	7.92
6.35	89.0	8.30
6.55	224.0	8.40
6.85	56.4	8.10
5.60	287.7	7.60
5.30	246.4	8.10
5.60	495.0	8.30
5.12	90.0	7.48
5.80	172.2	7.35
6.45	159.0	7.30
6.10	89.0	7.35
6.10	171.1	7.75
5.92	340.7	7.15
5.70	77.7	7.40
	70.3	7.82
	161.6	7.50
Av. 5.97	Av. 182.9	Av. 7.80

fecal fat than the normal subject, presumably because of the widely different diets of the subjects. Tables I and II summarize the experimental findings.

Aluminum hydroxide apparently caused a small but definite increase in urinary pH. The normal subject showed an average urine pH of 5.51 for the control period, and 5.72 for the aluminum hydroxide period. The ulcer patient showed values of 5.88 and 5.97 respectively. A much more marked effect of aluminum hydroxide upon total titratable urinary acidity was observed in both subjects. The normal subject showed an average 24-hour titratable urine acidity of 445 cc of 0.1 N acid during the control period, which dropped to 241 cc during the aluminum hydroxide period. The corresponding values for the ulcer patient were 265 cc and 183 cc respectively. The decrease in urinary acidity under the influence of aluminum hydroxide ingestion was probably due to a decrease in the excretion of urinary phosphate. Fauley, Freeman, Ivy, et al (8) have shown that urinary phosphate excretion in humans on light ulcer diets was decreased about 60 percent when aluminum hydroxide was given orally. On the other hand, fecal phosphate excretion greatly increased as the result of the formation of insoluble aluminum phosphate. Evidence was presented that much of this fecal phosphate had been removed from the body, leading to a phosphate deficiency. According to these facts we believe that the removal of phosphate ions from the blood by aluminum hydroxide therapy should be reflected in a somewhat higher urine pH and a lowered titratable acidity. Our findings on humans agree with this interpretation.

The average fecal pH value of the normal subject for the control and aluminum hydroxide periods were 5.99 and 6.69 respectively, while the values for the ulcer patient were 8.06 and 7.8. Tables III-IV give the experimental result on acidity of urine and feces.

Summary: Administration of aluminum hydroxide to a normal subject did not interfere with the utilization of carbohydrate, fats, or proteins of the diet. Apparently there was no interference with carbohydrate and fat utilization in a gastric ulcer subject, but results on protein utilization were inconclusive.

Aluminum hydroxide administration caused slight increases in urinary pH and very definite decreases in total urinary acidity.

REFERENCES

1. Komarov, S. A. and Krueger, Luise. The Effect of Aluminum Hydroxide Gel on Gastric Secretion in the Dog. Am. J. Dig. Dis., 7:170, 1940.
2. Komarov, S. A. and Komarov, Olga. The Precipitability of Pepsin by Colloidal Aluminum Hydroxide. Am. J. Dig. Dis., 7:166, 1940.
3. Schiffrin, M. J. and Komarov, S. A. The Inactivation of Pepsin by Compounds of Aluminum and Magnesium. Am. J. Dig. Dis., 8:215, 1941.
4. Beazell, J. M., Schmidt, C. R. and Ivy, A. C. The Effect of Aluminum Hydroxide Cream on Absorption from the Gastrointestinal Tract. Am. J. Dig. Dis., 5:164, 1939.
5. Steiner, A., Urban, F. and West, E. S. Iron and Thorium Precipitation of Biological Fluids for Sugar and other Analyses. J. Biol. Chem., 98:289, 1932.
6. Shaffer, P. A. and Somogyi, M. Copper-iodometric Reagents for Sugar Determination. J. Biol. Chem., 100:695, 1933.
7. Saxon, Gordan J. A Method for the Determination of the Total Fats of Undried Feces and Other Moist Masses. J. Biol. Chem., 17:99, 1914.
8. Fauley, G. B., Freeman, S., Ivy, A. C., Atkinson, A. J. and Wigodsky, H. S. Aluminum Phosphate in the Therapy of Peptic Ulcer. Arch. Int. Med., 67:563, 1941.

Soil Sci. 60: 29-40 (1945)

ALUMINUM IN SOILS, PLANTS, AND ANIMALS

G. EVELYN HUTCHINSON[1]

Osborn Zoological Laboratory, Yale University

Aluminum occupies a somewhat anomalous position among the biological elements, in that it is a very common and important constituent of the inorganic materials of the biosphere, but a rare and usually unimportant constituent of living matter itself. Both the importance of the element in the environment and its relative unimportance within the organism are ultimately to be referred to the dimensions and charge of the Al^{+++} ion.

The radius of the aluminum in ionic crystals falls between that of the doubly charged magnesium and the quadruply charged silicon ion: Mg^{++}, $r = 0.78$; Al^{+++}, 0.57; Si^{++++}, 0.39. In the lattice structure of the aluminosilicate clay minerals, limited substitution of Al^{+++} for Si^{++++} or of Mg^{++} for Al^{+++} is possible. The base-exchange properties of the pedolites are now frequently interpreted in terms of the lack of electrostatic balance produced by such substitutions.

The relative unimportance of aluminum within the organism is doubtless due to the low solubility of the element in neutral solutions. Goldschmidt (14) has pointed out that, considering only those lithophil elements of essentially invariant valency, a diagram may be constructed in which the point occupied by the element is defined by its ionic radius and its valency (fig. 1).

Elements having a low ratio of charge to radius tend to form more or less soluble cations; elements having a high ratio form anions with oxygen. In the middle sector of the quadrant, in which Be, Al, Ti, Th, Zr, and Hf lie, the elements tend to form oxides insoluble in neutral water. The contours introduced into the diagram express the ratio of mean concentration within living plants to that in the accessible lithosphere and so indicate the importance of the relationship in determining biological availability. As the commonest of the elements occupying the middle sector of this diagram, the behavior of aluminum has a theoretical interest in biogeochemistry, while the definition of the conditions under which abnormal amounts of the element are present in the immediate

[1] Thanks are due W. O. Robinson, S. B. Hendricks, and R. A. Steinberg for reading the manuscript and for offering helpful comments.

Since this article was written, a paper by B. B. Polynov [*Bull. Akad. Nauk. USSR, Ser. Geol.* 1944 (2) 3–14] has been received. The author notes considerable amounts of aluminum in all plants growing on alluvial red earth in the foothills of the Caucasus. *Betula betula* contained 8.5 per cent Al in the ash of the leaves, whereas in other parts of Europe only a trace is present. He regards aluminum accumulation as a region phenomenon dependent on climatically determined soil types; it is, however, clear that the vegetation of such regions consists of aluminum-tolerant species. Polynov also indicates that he has found that the lichen *Parmelia* promotes the decomposition of primary rocks; both Al_2O_3 and SiO_2 enter the plant body, and on subsequent death and decay montmorillonite is formed.

environment of organisms is of great practical agricultural importance. The present review aims at summarizing the present state of knowledge of all the more interesting aspects, both theoretical and practical. Since the writer has recently published a longer discussion of the same subject (22), bibliographic citation to older work, now superseded or known to be erroneous, will not be given in the present contribution.

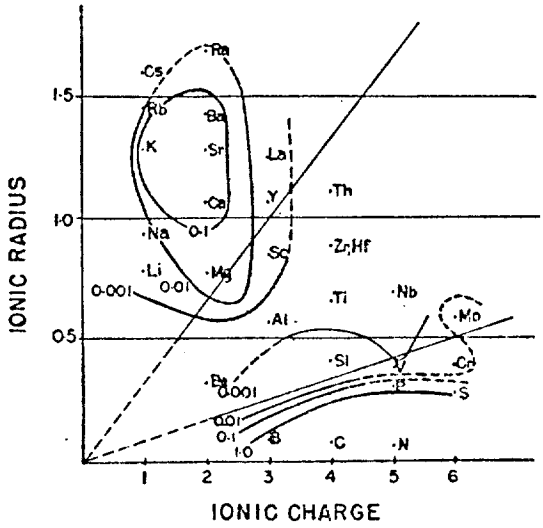


FIG. 1

ALUMINUM IN SOILS

Aluminum occurs in undecomposed rock fragments, in secondary alumino-silicate clays, as solid or hydrosol hydrated oxides, as phosphates, and in ionic form. The decomposition of rock fragments is a much-studied but inadequately understood process. Mechanical subdivision and the action of colloidal clays and organic matter may remove cations and so initiate decomposition. Carbon dioxide and organic acids have generally been regarded as important agents; the role of sulfuric acid has been somewhat neglected. The origin of alumina in soils may be attributed to such decomposition, or to the similar decomposition of secondary minerals. Vinogradov and Boichenko (67) have established that diatoms can decompose nacrite, and therefore biological action in liberating alumina has to be considered. The general trend of such changes has long been known to depend on climate, sesquioxides migrating downward in podzols of high-temperate latitudes, whereas silica is removed by leaching in the tropics. Even in low-temperate latitudes *gibbsite* $Al(OH)_3$ may constitute 30 per cent of the inorganic soil colloid (1).

The phosphate present in virgin soils varies considerably in availability and when relatively soluble phosphatic fertilizers are added to soils, the additional phosphate tends to become fixed in a more or less insoluble form. Fixation by

formation of aluminum and iron phosphates, and by aluminosilicate pedolites occurs, but in most cases the former process is of somewhat greater importance (6).

Little information exists as to the mineralogy of the aluminum phosphate in soils. Various attempts have been made to identify mineral phosphates by means of pH solubility curves. Stelly and Pierre (61), however, find that mixtures of apatite and vivianite cannot be satisfactorily distinguished from aluminum phosphates, nor can the two aluminum phosphates most likely to occur, namely, variscite, $AlPO_4 \cdot 2H_2O$, and wavellite, $Al_3(OH)_3(PO_4)_2 \cdot 5H_2O$, be separated by their solubility minima, though the basic wavellite is somewhat more soluble under the conditions of their experiments. Their work, however, gives strong evidence for the existence of different mineral phosphates in different soils.

A great variety of complex aluminum phosphates is known from sedimentary rocks; some of these might be soil constituents. The clay-like minervites, supposed by Vernadsky (66) to be analogous to the aluminosilicate clays, deserve renewed investigation.

The occurrence of aluminum extracts of acid soils made with neutral salt solutions has been known at least from the observations of Veitch (65). Rice (45) showed that the element did not appear until the pH of the extract fell below 4.5. The whole question became of considerable practical importance when Hartwell and Pember (17) obtained evidence that the injurious effects of acid soils on certain plants was due to aluminum rather than to hydrogen ions. Subsequent work, however, has indicated that the matter is far from simple.

Except in the presence of sulfuric acid, it is probable that hydrosols of hydrated alumina are of general occurrence. Joffe and McLean (24) find that this colloidal aluminum may be in excess of the aluminum in the solution. The origin of aluminum appearing in solution in the aqueous phase of acidified soils is no doubt in part from solution of hydrated oxide, but there is evidence that so-called hydrogen-saturated clays are partly saturated with aluminum ions derived from lattice positions (2, 15).

Mattson (35) points out that when soils are electrodialyzed, a considerable amount of silica appears at the cathode. This must be transported as complex aluminosilicate cations. Mattson believes that Al^{+++} ions appear only at very low pH values, and that normally aluminosilicate and aluminohydroxyl ions, $Al(OH)^{++}$, are the chief ionic forms of aluminum in the soil solution. More recently Gapon and Voshchinskaya (11, 12) have concluded that in a certain podzol soil which they studied, 90 per cent of the soluble aluminum was present as oxyaluminum ions, $Al_2O_3H^+$. Treatment of a KCl extract of such a soil with ammonium sulfate produces a precipitate of aluminum oxysulfate, $(Al_2O_3H)_2SO_4$. The remaining 10 per cent of the soluble aluminum is referred to colloidal alumina and Al^{+++}. The possible existence of complex ions of aluminum and organic acids has been mentioned. It is clear that even the soluble aluminum of soils can take very many forms and that our knowledge of the equilibriums involved is quite inadequate. The matter is, however, of great biological importance, as is easily appreciated when the great toxicity of the element in

aqueous culture solutions is compared with the reduced toxicity of the element in soil cultures, as judged from the aluminum concentration in displaced soil solutions. Gapon and Voshchinskaya (11) at least concluded that aluminum as $Al_2O_3H^+$ was far less toxic than as Al^{+++}, as indeed might be expected. It must also be remembered that aluminum is mobile in alkaline soils, and that since aluminum phosphate can be a source of phosphorus around neutrality, some aluminum can doubtless enter some plants at any pH.

ALUMINUM IN VASCULAR PLANTS

Mean content

With sufficiently refined methods, aluminum can apparently always be detected in the tissues of flowering plants. The most reliable data for herbaceous vegetation (47, 30, 58) indicate a mean content of about 0.02 per cent Al in the dry matter. Robinson *et al.* (47) found a like content in woody parts, but the spectrographic studies of de Rubies and Lemmel (48) perhaps indicate that such an estimate for wood is too high. On the basis of all these data, a mean value of 0.002 per cent Al in the living plant seems reasonable.

Accumulator species

Certain species habitually accumulate amounts of aluminum vastly in excess of this small though variable amount of the element normally present [see Hutchinson (22) for full discussion]. The most remarkable cases are found in the Proteaceous tree, *Orites excelsa* R. Br., in which a deposit of a basic aluminum succinate, $Al_2(C_4H_4O_4)_3Al_2O_3$, in a cavity in the trunk is recorded (59); in various Theaceae (10, 68); in most Melastomaceae (10, 16, 23); in the Euphorbiaceous genera, *Baccaurea* and *Aporosa* (16, 23); in hickory, *Hicoria ovata* (46); in the whole family Diapensiaceae (23, 68); and in *Symplocos* (8, 10, 23, 26, 44, 46, 49, 68). Many of these cases have been discovered through the use of the plant as a source of mordant in the traditional technology of dyeing. In some species of *Symplocos*, cell inclusions occur which were supposed by Radlkofer to contain aluminum; Kratzmann, however, finds that after ashing, they are insoluble in HCl though soluble in HF. It is just possible that these inclusions contain an aluminosilicate of biological origin; in view of the demonstration by Vinogradov and Boichenko that nacrite can be hydrolyzed biologically, any case of possible aluminosilicate synthesis in an organism acquires great potential interest.

DISTRIBUTION OF ALUMINUM IN PLANT BODY

A number of authors have concluded that there is a gradient in aluminum content, the amount falling off from the root to the leaf. This is certainly true in a number of species growing on very acid soil, the most extreme cases being the solfatara plants described by von Faber (10), in which accumulation occurs in the root, but not necessarily in the aerial parts. In more normal plants the leaf content of aluminum, as of other ash constituents, is often greater than that of the stem (28, 58).

ALUMINUM IN PTERIDOPHYTES

Though the recent work of Tauböck, discussed hereinafter, apparently indicates aluminum to be an essential element in the nutrition of Pteridophytes, the few reliable analyses of ferns indicate that most species have no greater content of the element than do normal flowering plants (5, 30). There are, however, a number of microchemical qualitative determinations by Kratzmann (26) which suggest that when an adequate number of ferns has been analyzed the amounts of aluminum present will prove in general greater than in the spermatophytes. Two groups of Filicales appear to be undoubted accumulators. The Australian ferns, *Platycerium grande* and *P. alcicorne*, which are usually epiphytes, contain 3.9 to 5.5 per cent Al in the ash or 0.18 to 0.38 per cent Al in the dry plant (7). A number of species of tree ferns of the family Cyatheaceae are aluminum plants (5, 68); good analytical data, on taxonomically determined specimens, are restricted to Tauböck's record of 6.2 per cent Al in the ash of *Alsophila australis*, though in culture the supply could be reduced to an undetectible amount without injury to the plant.

The few species of Equisitales that have been adequately studied, and *Selaginella* and *Psilotum* among the Lycopodiales, contain little aluminum, but in *Lycopodium* itself very marked accumulation has been known for over a century; the available information has been summarized by Hutchinson and Wollack (23). Some degree of taxonomy on the basis of ash composition is obviously possible in *Lycopodium* and the interest of the case is greatly increased by the fact that Manske and his associates (34) are building up a far more refined and quite independent chemical taxonomy based on the alkaloids found in these plants. A particularly interesting feature of the aluminum accumulation by *Lycopodium* is its specificity. Hutchinson and Wollack found no excessive accumulation of either iron or gallium in *L. flabelliforme*, and also believed, though on not quite adequate analytical evidence, that the rare earth content of the plant is very low. This suspicion has been confirmed by Robinson, as is indicated in his contribution to the present series of papers. In this specificity the accumulating mechanism appears to differ from that in the hickory and probably that in *Symplocos* also. The low iron content is in marked contrast to cases where pathological accumulation of both iron and aluminum in diseased corn stalks has been described (18, 19).

METABOLIC SIGNIFICANCE OF ALUMINUM IN PLANTS

The most interesting of the older experiments apparently indicating a metabolic role for the element is that of Sommer (60), who found pronounced stimulation, particularly indicated in the seed yield, on addition of 1 mgm. Al per liter to solution cultures supposedly free from the element. These experiments and some similar but much less striking observations of Lipman (32) on maize, should be repeated, with Steinberg's criticism in mind that the observed effects may be due to the accidental correction of a gallium deficiency.[2]

[2] Gallium-free $AlCl_3$ solution is easily prepared by extraction of the gallium with ether from a solution in 6 *N* HCl.

Tauböck (63) grew 124 species of flowering plants in solution culture. Spectrographic control of his medium indicated that it contained less than 25 γ Al per liter. He observed no symptoms of aluminum deficiency, and in eight species a second generation was successfully reared from seed set by the first. It must, however, be pointed out that the quantities of minor elements (boron, manganese, copper, zinc) added in Tauböck's experiments were of the same order of magnitude as the upper limit of Al concentration possible. Moreover, it was not practicable to avoid uptake of Al from dust through the epidermis of the aerial parts of the plant.

Although these and other experiments leave the metabolic role of aluminum in seed plants of normal composition somewhat uncertain, a few observations have been published suggesting that the element is of importance to accumulator species.

Symplocos japonica, according to Neger (41), grows much better in Knopf's solution containing 1.0 mgm.-atom Al per liter than in solutions containing 0.0, 0.1, or 10.0 mgm.-atoms. From all solutions, including the control, the plants acquired some microchemically detectible aluminum, but the uptake was greatest from the optimal concentration. No indication of control of acidity is, however, given in the description of the experiments.

Vaccinium varingiifolium, *V. lucidum*, *Rhododendron retusum*, and *Ficus diversifolia*, growing in certain excessively acid solfatara soils in Java, were found by von Faber (10) to require aluminum for normal growth, though in these plants accumulation of the element is confined to the roots. At least in the case of *F. diversifolia*, the control culture was acidified, and therefore one criticism that might be brought against Neger's experiment on *Symplocos* is excluded. Comparable experiments were performed by Yoshii (69) with like results.

Turning to the Pteridophytes, we find according to Tauböck a very different situation, for here both the accumulator species, *Alsophila australis* and two species of more normal composition, namely, *Aspidium Filix-mas* and *Polypodium proliferum*, all failed to develop normally unless 0.16 mgm. Al per liter was added to the buffered culture solution. Tauböck's observations on these ferns are in line with the results of Kratzmann (27) who, in somewhat ill-controlled experiments, found that spores of *Equisetum arvense* failed to produce normal gametophytes unless aluminum nitrate were present in the medium. A particularly interesting aspect of Tauböck's experiment is that although *Alsophila* required aluminum for normal development, the quantity needed is much less than that required to produce the aluminum accumulation apparently normal to the plant when growing in soil. The parent tree-fern from which Tauböck obtained his spores contained 6.2 per cent Al in the leaf ash, whereas his plants, though permitted to grow in culture in the presence of 0.16 mgm. Al per liter, failed to accumulate the element. It would appear that a mechanism has been developed to permit the entry of the element, but not to regulate the excess consumption of the large quantities normally present in the environment. This, however, can hardly be true of the other ferns that he studied, for in *Aspidium Filix-mas* Church (5) found only traces of aluminum, though Stoklasa (62) notes, probably

quite unreliably, 0.51 per cent Al in the ash of the aerial parts. Conceivably such species may have developed a more accurate regulatory mechanism, though, as has been noted, it is possible that the aluminum content of ferns is somewhat higher than of flowering plants. It is, moreover, doubtful whether mere excess consumption will explain the taxonomic regularity of accumulation in *Lycopodium*, where it is reasonably certain that the high capacity to accumulate the element, often in rather constant amounts, represents an evolutionary specialization.

All these experiments can be criticized on the grounds that gallium may be involved as an impurity in the aluminum salts used, and all should be repeated with such a thought in mind. In the case of *Symplocos*, experiments should also be conducted with the rare earth elements. Tauböck's work at least indicates unequivocably that the three ferns studied require aluminum, or some element associated with aluminum, in much greater quantity than do most of the flowering plants. To the present writer, who is possibly overimpressed by the specificity of the aluminum-accumulating mechanism in *Lycopodium*, the most reasonable explanation of the observations is that aluminum is actually required by the ferns and probably by the other vascular cryptogams.

Apart from these experiments mention may be made of work by Sergeiev and Sergeieva (56, 57), which appears to indicate an increase in the frost resistance of wheat, due to aluminum, and of the remarkable observations of Liebig, Vanselow and Chapman (31), demonstrating that aluminum protects citrus cuttings from the poisonous effects of a slight excess of copper. The presence of aluminum had no effect on the distribution of copper in the plant, nor was any evidence of precipitation of copper by the aluminum in the culture medium to be noted. The well-known effect of aluminum in the production of blue flowers in *Hydrangea* has been the subject of many investigations, admirably reviewed by Chenery (4), who showed that the blue color is due to formation of an aluminum complex of a delphinidine diglycoside.

EXCESS ALUMINUM AS AN ECOLOGICAL FACTOR

By far the most important aspect of aluminum is its action, when present in excess in soluble form, as a limiting factor to the growth of certain plants. This aspect of the biogeochemistry of the element was first clearly indicated by Ruprecht (50), Ruprecht and Morse (51, 52), and Hartwell and Pember (17). An immense amount of work has been done on the problem, and it would now appear that acid soils are unsuitable for some plants on account of their acidity, but that, in other cases, the increasing concentration of hydrolyzate-oxidate elements limits the growth of plants. In view of the number of contributions that have appeared on this matter, a synoptic summary of results alone can be attempted in the present review. A full summary of the literature has been given in an earlier review (22).

In a number of cases amounts of aluminum, as Al^{+++}, of the order of 1 mgm. per liter exercise a distinctly unfavorable effect in solution cultures.

There is much interspecific variation in the threshold or fatal doses of aluminum; Gilbert

and Pember (13) have adduced evidence that such variation in tolerance may regulate competition between certain grasses and dicotyledons.

There is a great discrepancy between the threshold toxicity of Al^{+++} in solution culture and the amount present in soil solutions in pot cultures of plants just showing injury. Thus Sommer (60) found 2 mgm. Al per liter injurious to wheat in solution culture, whereas Mattson and Hester (36) did not get an effect with two electrodialyzed soils adjusted to various pH values until 1 or 2 mgm. soluble (Al, Fe_2O_3 per 100 gm. soil were present, corresponding to at least 5 to 10 mgm. Al per liter of soil solution and probably considerably more. Mattson and Hester also found that silication of a soil not merely reduced the pH at which a given amount of aluminum appeared in solution but also increased the tolerance to such a given concentration. The form in which aluminum is present in the soil solution is clearly of great importance. Mattson and Hester have given evidence that alumino-silicate ions exist and believe them less toxic than Al^{+++}. Gapon and Voshchinskaya (11, 12), as has already been indicated, found evidence of $Al_2O_3H^+$ ions, which they concluded are relatively nontoxic.

Phosphate treatment and liming both reduce the aluminum toxicity of a soil. In the case of lime treatment, presumably a rise in pH causes less aluminum to go into solution. In the case of phosphate treatment the mode of action is obscure. Burgess and Pember (3) and Pierre and Stuart (42) found that addition of phosphate did not cause any decrease in the aluminum uptake but did cause a marked increase in phosphate uptake. It is possible that the aluminum is fixed as the relatively insoluble $AlPO_4$ within the plant, particularly in the roots. Some workers, notably Sergeiev and Sergeieva (56, 57), believe that there is a specific ionic antagonism between Al^{+++} and PO_4^{---}, but the evidence cannot be regarded as satisfactory. The experiments of Trenel and Alten (64) are of considerable interest. In one series, corn plants with divided roots were exposed on one side to a complete nutrient solution without aluminum, and on the other, to aluminum solution. Injury was limited to the roots exposed to aluminum. In another series, aluminum was added to a phosphate-free nutrient solution on one side, and phosphate supplied on the other. Here the whole plant was inhibited. The percentage of N, K, Ca, and P in the dry matter of such plants was, however, greater than in the controls, so that these elements were taken up by the injured roots in excess of the amount the depauperated plant could effectively utilize. In most cases, however, the relative magnesium content was reduced, but one series failed to show this effect. It is tempting to suppose that the root injury may limit uptake and utilization of magnesium and therefore assimilatory processes, but the exceptional case probably excludes such an interpretation. Interference with normal copper metabolism may conceivably have to be considered in view of the results of Liebeg, Vanselow, and Chapman (31).

ALUMINUM IN ANIMALS

Most of the aluminum taken up by herbivorous animals is apparently excreted with the feces; at least a part of this has apparently been absorbed and passed into the bile. The net result of the assimilatory process is, however, to produce an organism considerably poorer in aluminum than is the food. The best results (9, 29, 37, 38, 39) indicate that whereas mammalian muscle is very low in the element, the viscera may contain a little over 1 mgm. Al per kilogram wet. Myers and Mull (40) and Kehoe, Cholak, and Story (25) find like amounts in man; Lundegårdh and Bergstrand (33), investigating many human livers, however, found a mean value twenty or thirty times greater than that of other investigators on man or other mammals. The explanation of this discrepancy is not clear, as Lundegårdh and Bergstrand's results for other elements agree with those of previous investigators. For the present the best general value for

mammalian tissue is probably about 0.5 mgm. per kilogram, representing about one fortieth of the quantity of the element in the food. Scoular (55) found no evidence of retention of aluminum in the growing human child, though she (53, 54) was able to detect retention of zinc and copper. Despite the low quantity of aluminum present in mammalian tissue and the negative results of all modern experiments producing aluminum-deficiency symptoms in mammals, there is evidence from the work of Horecker, Stotz, and Hogness (20) and of Potter and Schneider (43) that aluminum is involved in the succinic dehydrogenase-cytochrome C system, which is the chief mechanism for oxidation of succinate in the mammalian body. It is supposed that aluminum is involved in the reaction between cytochrome-C and its reductant, presumably succinic dehydrogenase. Some other tervalent elements, namely, Cr, Nd, La, and Sm, can substitute for aluminum. Analytical evidence, both from the enzyme preparations themselves and from the known composition of the mammalian body, strongly indicates that aluminum is actually the element involved *in vivo*. It is noteworthy that on the diet containing the least amount of aluminum yet available, which supplied 1 to 1.5 γ daily to the rats used in the experiment, the liver accumulated 90 to 107 γ per kilogram of the element, and that increasing the aluminum in the diet twentyfold to thirtyfold caused but a threefold increase in the liver aluminum (21).

Finally it is desirable to point out briefly that although massive doses of aluminum in stoichiometric excess of the phosphate intake may cause a rachitic condition, there is little evidence that this would ever happen from ingestion of human food prepared from aluminum-containing baking powders. The supposed injurious effect of aluminum cooking vessels appears to be quite apocryphal. The most striking species of accumulator plants, however, certainly contain a great excess of aluminum over phosphate, and would constitute by themselves impossible diets for herbivorous vertebrates unless, as is very unlikely, the aluminum is in some form incapable of reacting with phosphate prior to absorption of the latter in the intestine.

REFERENCES

1. Alexander, L. T., Hendricks, S. B., and Faust, G. T. 1941 Occurrence of gibbsite in some soil forming materials. *Proc. Soil Sci. Soc. Amer.* 6: 52–57.
2. Allen, F., and Ikrammet, B. 1935 Die physikalisch-chemischen Gesetzmässigkeiten beim Kationenaustausch im Mineralboden. *Ernähr. Pflanze* 31: 401–407.
3. Burgess, P. S., and Pember, F. R. 1923 Active aluminum as a factor detrimental to crop production in many acid soils. R. I. Agri. Exp. Sta. Bul. 194.
4. Chenery, E. M. 1937 The problem of the blue hydrangea. *Jour. Roy. Hort. Soc.* 62: 304–320.
5. Church, A. H. 1888 On the occurrence of aluminium in certain vascular cryptogams. *Proc. Roy. Soc. [London]* 44: 421–429.
6. Coleman, R. 1943 The adsorbition of phosphate by kaolinitic and montmorillonitic clays. *Proc. Soil Sci. Soc. Amer.* 7: 134–138.
7. Dixon, W. A. 1881 On the inorganic constituents of some epiphytic ferns. *Jour. Roy. Soc. N.S. Wales* 15: 175–183.
8. Driessen, F. 1902 Étude sur le rouge turc ancien procédé. *Rev. Gén. Mat. Color.* 6: 252–259. (Reprint from Bul. Soc. Indus. Mulhouse 180, 1902.)

(9) EVELETH, D. F., AND MYERS, V. C. 1936 Studies on aluminum: II. The storage of intravenously injected aluminum in the dog. *Jour. Biol. Chem.* 113: 467-471.
(10) FABER, F. C. VON 1927 Die Kraterpflanzen Javas in physiologisch-ökologischer Beziehung. Arb. Treub-Lab., Weltevreden 1.
(11) GAPON, E. N., AND VOSHCHINSKAYA, M. A. 1941 Exchangeable oxy-aluminum cations in podzolic soils. *Dokl. Akad. Selsk. Khoz. Nauk* 5: 46-48.
(12) GAPON, E. N., AND VOSHCHINSKAYA, M. A. 1941 The oxyaluminum of podzolized soils as an exchangeable cation. *Chemization Socialistic Agr.* (U.S.S.R.) 10 (2): 37-42. (Original not seen. Abstract in *Chem. Abs.* 37: 489.)
(13) GILBERT, B. E., AND PEMBER, F. R. 1935 Tolerance of certain weeds and grasses to toxic aluminum. *Soil. Sci.* 39: 425-430.
(14) GOLDSCHMIDT, V. M. 1937 The principles of distribution of chemical elements in minerals and rocks. *Jour. Chem. Soc.* 140: 655-673.
(15) GRIM, R. E. 1942 Modern concepts of clay materials. *Jour. Geol.* 50: 225-275.
(16) HALLIER, H. 1922 Beiträge zur Kenntnis der Linaceae (DC. 1819) Dumort. *Beihefte Bot. Centbl.* 39 (abt. 2): 1-178. (*Vide* section 18. Die Pentaphylaceen und Aluminiumpflanzen, pp. 128-137.)
(17) HARTWELL, B. L., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *Soil Sci.* 6: 259-281.
(18) HOFFER, G. N., AND CARR, R. H. 1923 Accumulation of iron and aluminum compounds in corn plants and its probable relation to root rots. *Jour Agr. Res.* 23: 801-825.
(19) HOFFER, G. N., AND TROST, J. F. 1923 The accumulation of iron and aluminum compounds in corn plants and its probable relation to root rots: II. *Jour. Amer. Soc. Agron.* 15: 323-331.
(20) HORECKER, B. L., STOTZ, E., AND HOGNESS, T. R. 1939 The promoting effect of aluminum, chromium, and the rare earths in the succinic dehydrogenase-cytochrome system. *Jour. Biol. Chem.* 128: 251-256.
(21) HOVE, E., ELVEHJEM, C. A., AND HART, E. B. 1938 Al in the nutrition of the rat. *Amer. Jour. Physiol.* 123: 640-643.
(22) HUTCHINSON, G. E. 1943 The biogeochemistry of aluminum and of certain related elements. *Quart. Rev. Biol.* 18: 1-29, 128-153, 242-262, 331-363.
(23) HUTCHINSON, G. E., AND WOLLACK, A. 1943 Biological accumulators of aluminum. *Conn. Acad. Arts and Sci. Trans.* 35: 73-128.
(24) JOFFE, J. S., AND MCLEAN, H. C. 1928 Colloidal behavior of soils and soil fertility: V. The distribution of soluble and colloidal iron and aluminum in soils. *Soil Sci.* 26: 317-326.
(25) KEHOE, R.A., CHOLAK, J., AND STORY, R. V. 1940 A spectrochemical study of the normal ranges of concentration of certain trace elements in biological materials. *Jour. Nutrition* 19: 579-592.
(26) KRATZMANN, E. 1913 Der mikrochemische Nachweis und die Verbreitung des Aluminiums im Pflanzenreich. *S.B. Akad. Wiss. Wien. Math. Nat. Kl.* 122, Abt. 1 (1-4): 311-336.
(27) KRATZMANN, E. 1914 Zur physiologischen Wirkung der Aluminiumsalze auf die Pflanze. *S.B. Akad. Wiss. Wien. Math. Nat. Kl.* 123, Abt. 1 (1-5): 221-239.
(28) LATSHAW, W. L., AND MILLER, E. C. 1924 Elemental composition of the corn plant. *Jour. Agr. Res.* 27: 845-861.
(29) LEHMANN, K. B. 1931 Aluminium Bestimmung und Aluminiumgehalt von Nährungsmitteln. *Arch. Hyg., Berlin* 106: 309-335.
(30) LEVY, G. 1931 La présence, la répartition et la rôle de l'aluminium chez les végétaux. Paris. (Thesis.)
(31) LIEBIG, G. F., VANSELOW, A. P., AND CHAPMAN, H. D. 1942 Effects of Al on Cu toxicity as revealed by solution-culture and spectrographic studies of citrus. *Soil Sci.* 53: 341-352.

(32) LIPMAN, C. B. 1938 Importance of silicon, aluminum, and chlorine for higher plants. *Soil Sci.* 45: 189–198.

(33) LUNDEGÅRDH, H., AND BERGSTRAND, H. 1940 Spectral-analytical investigations into the content of mineral substances in the liver. *Nova Acta Soc. Sci. Upsala* (Ser. IV) 12: no. 3.

(34) MANSKE, R. H. F., AND MARION, L. 1942–1944 The alkaloids of *Lycopodium* species: I, II, III, IV. *Canad. Jour. Res* (B) 20: 87–92, 153–156, 1942; 21: 92–96, 1943; 22: 1–4, 1944.

(35) MATTSON, S. 1933 The laws of soil colloidal behavior: XI. Electrodialysis in relation to soil processes. *Soil Sci.* 36: 149–162.

(36) MATTSON, AND HESTER, J. B. 1933 The laws of soil colloidal behavior: XII. The amphoteric nature of soils in relation to Al-toxicity. *Soil Sci.* 36: 229–244

(37) MEUNIER, P. 1936 Sur la présence et la répartition de l'aluminium dans les tissus animause. *Compt. Rend. Acad. Sci.* [*Paris*] 203: 891–894.

(38) MYERS, V. C., AND MULL, J. W. 1928 The influence of the administration of aluminum upon the aluminum content of the tissues and upon the growth and reproduction of rats. *Jour. Biol. Chem.* 78: 605–613.

(39) MYERS, V. C., AND MORRISON, D. B. 1928 The influence of the administration of aluminum upon the aluminum content of the tissues of the dog. *Jour. Biol. Chem.* 78: 615–624.

(40) MYERS, V. C., AND MULL, J. W. 1928 The aluminum content of human autopsy tissue. *Jour. Biol. Chem.* 78: 625–626.

(41) NEGER, F. W. 1923 Neue Methoden und Ergebnisse der Mikrochemie der Pflanzen. *Flora* 116 (n.f. 16): 323–330.

(42) PIERRE, W. H., AND STUART, A. D. 1933 Soluble Al studies: IV. The Effects of P in reducing the detrimental effects of soil acidity on plant growth. *Soil Sci.* 36: 211–228.

(43) POTTER, V. R., AND SCHNEIDER, W. C. 1942 Studies on the mechanism of hydrogen transport in animal tissues: V. Dilution effects in the succinoxidase system. *Jour. Biol. Chem.* 142: 543-555.

(44) RADLKOFER, L. 1904 Über Tonerdekörper in Pflanzenzellen. *Ber. Deut. Bot. Gesell.* 22: 216–224.

(45) RICE, F. E. 1916 Studies in soils: I. *Jour. Phys. Chem.* 20: 214–227.

(46) ROBINSON, W. O. 1938 The agricultural significance of the minor elements. *Amer. Fert.* 89 (8): 5-8, 24, 26.

(47) ROBINSON, W. O., STEINKOENIG, L. A., AND MILLER, G. F. 1917 The relation of some of the rarer elements in soils and plants. U. S. Dept. Agr. Bul. 600.

(48) RUBIES, P. DE, AND LEMMEL, L. 1935 Étude spectroscopique de différents bois de Fernando Po et de la Péninsule Ibérique. *Bul. Soc. Chim. France* (5) 2: 1368–1370.

(49) RUMPHIUS, G. E. 1743 (Ed. Joannes Burmannus) Herbarium Amboinense (Het Amboinsch Kruid-boek) Pars. tertia. Amsterdam, Hague, and Utrect.

(50) RUPRECHT, R. W. 1915 Toxic effect of iron and aluminum salts on clover seedlings. *Mass. Agr. Exp. Sta. Bul.* 161: 125–129.

(51) RUPRECHT, R. W., AND MORSE, F. W. 1915 The effect of sulfate of ammonia on soil. *Mass. Agr. Exp. Sta. Bul.* 165: 73–90.

(52) RUPRECHT, R. W., AND MORSE, F. W. 1917 The cause of the injurious effect of sulphate of ammonia when used as a fertilizer. *Mass. Agr. Exp. Sta. Bul.* 176: 119–134.

(53) SCOULAR, F. I. 1938 A quantitative study, by means of spectrographic analysis, of copper in nutrition. *Jour. Nutrition* 16: 437–450.

(54) SCOULAR, F. I. 1939 A quantitative study, by means of spectrographic analysis, of zinc in nutrition. *Jour. Nutrition* 17: 103–113.

(55) SCOULAR, F. I. 1939 A quantitative study, by means of spectrographic analysis, of Al in nutrition. *Jour. Nutrition* 17: 393–405.

(56) SERGEIEV, L. I., AND SERGEIEVA, K. A. 1939 Effect of ions of aluminum and phosphoric acid on biological properties of plant protoplasm. *Compt. Rend. Acad. Sci.* [*U.R.S.S.*] 22: 626–629.

(57) SERGEIEV, L. I., AND SERGEIEVA, K. A. 1939 Ionic action as a means of controlling resistance and growth of plants. *Compt. Rend. Acad. Sci.* [*U.R.S.S.*] 22: 630–632.

(58) SHORLAND, F. B. 1934 The estimation of Al in pastures with special reference to soil contamination. *Proc. Roy. Soc. New Zeal.* 64: 35–50.

(59) SMITH, H. G. 1904 Aluminium, the chief inorganic element in a proteaceous tree and the occurrence of aluminium succinate in trees of this species. *Jour. Roy. Soc. N.S. Wales* (1903) 37: 107–120.

(60) SOMMER, A. L. 1926 Studies concerning the essential nature of aluminum and silicon for plant growth. *Univ. Calif. Pubs. Agr. Sci.* 5 (2): 57–81.

(61) STELLY, M., AND PIERRE, W. H. 1943 Forms of inorganic phosphorus in the C horizons of some Iowa soils. *Proc. Soil Sci. Soc. Amer.* 7: 139–147.

(62) STOKLASA, J. 1922 Über der Verbreitung der Aluminiums in der Natur und seine Bedeutung beim Bau- und Betriebstoffwechsel der Pflanzen. G. Fischer, Jena.

(63) TAUBÖCK, K. 1942 Über die Lebensnotwendigkeit des Aluminiums für Pteridophyten. *Bot. Arch.* 43: 291–304.

(64) TRENEL, M., AND ALTEN, F. 1934 Die physiologische Bedeutung der Mineralischen Bodenazidität (Worauf beruht die toxische Wirkung des Aluminiums?). *Angew. Chem.* 47: 813–820.

(65) VEITCH, F. P. 1904 Comparison of methods for the estimation of soil acidity. *Jour. Amer. Chem. Soc.* 26: 637–662.

(66) VERNADSKY, W. I. 1938 On the terrestrial alumophosphorous and alumosulphurous analogues of kaolinic alumosilicates. *Compt. Rend. Acad. Sci.* [U.R.S.S.] 18: 287–294.

(67) VINOGRADOV, A. P., AND BOICHENKO, E. A. 1942 Decomposition of kaolin by diatoms. *Compt. Rend. Acad. Sci.* [U.R.S.S.] 37: 135–138.

(68) YOSHII, Y., AND JIMBO, T. 1932 Mikrochemischer Nachweis von Aluminium und sein Vorkommen im Pflanzenreiche. *Tôhoku Imp. Univ. Sci. Rpts.* (Ser. 4), 7: 65–77.

(69) YOSHII, Y. 1937 Aluminum requirements of solfatara plants. *Bot. Mag.* [*Tokyo*] 51: 262–270. (Original not seen. Abstract in *Japan Jour. Bot.* 9: (97).)

SECTION I—*Clinical Medicine: Diseases of Digestion*

The Effect of Administration of Aluminum Preparations on the Secretory Activity and Gastric Acidity of the Normal Stomach*

By
A. C. IVY, M.D., LAWRENCE TERRY, M.S.,
G. B. FAULEY, M.D.
and
WM. B. BRADLEY, M.S.
CHICAGO, ILLINOIS

ALTHOUGH aluminum preparations have been and are being used to some extent in the treatment of "peptic" ulcer in man (1, 2, 3, 4, 5, 6), we have been unable to find a study of the effect of the prolonged administration of relatively large quantities of such preparations on the secretory activity of the stomach of normal subjects. Einsel, Adams and Myers (2, 3) observed that the gastric acidity of ulcer patients was reduced after several weeks of treatment with aluminum hydroxide cream ("Al cream"), although a normal histamine response was obtained. Such a reduction in acidity may be ascribed to several factors, such as: (a) a natural decline of the "abnormally reacting" stomach in consequence of the remission of the ulcer; (b) a decrease in gastric retention as the ulcer enters a remission; (c) the aluminum, because of an astringent action on the gastro-intestinal mucosa, or the coagulation of mucin, or a reduction in gastric digestion, may decrease the secretagogic action of food; (d) the aluminum may cause a mild gastritis. The absorption of aluminum in systemic toxic amounts and an effect on the acid-base balance of the blood has apparently been ruled out (3). Because the possible operation of the first two factors one cannot derive from Einsel's observations what the real effect of aluminum medication on gastric secretion is. This work was primarily undertaken to answer that question.

METHODS

The investigation was conducted chiefly on healthy, vigorous dogs fed a balanced diet ad libitum. Two aluminum preparations were used. One was aluminum hydroxide cream. (In the earlier part of the work the cream was made by us; later we purchased Creamalin (Cleveland Chemical Associates), the buffering action of which was almost the same as our's). The other was Alucol, a colloidal aluminum hydroxide (Wander Co.) in the form of a powder, which was administered with a little water. A few experiments have also been performed on human subjects primarily for the purpose of ascertaining the degree of buffering of gastric acidity obtained with the aluminum preparations.

The capacity of the two preparations to buffer N/10 HCl was determined. Neither was immediately soluble in a large excess of N/10 HCl, Alucol being considerably less so than the Al cream, a factor that is concerned in the interpretation of our results. The samples of the preparations were set aside in contact with excess acid for periods of ½, 1, and 2 hrs., and then back-titrated with N/10 NaOH electrometrically. In order for one to check our results the details of our method of titrating must be given. Samples of 2 c.c. of the Al cream and 1 gram of Alucol powder were used. The samples were placed in 100 c.c. of N/10 at 37° C., the mixture being stirred about ¼ min. every 5 min. At the end of the periods stated, the samples were quickly cooled to room temperature (20° C.), 5 drops of 0.2% Töpfer's reagent were added and the mixture back-titrated with N/10 NaOH. Readings of alkali used were made at three different H-ion concentrations, namely, pH 3.76, or the first indication of change of Töpfer's from the red, at pH 4.0, and at the change of Töpfer's reagent to yellow, or a pH of 4.2. One c.c. of aluminum hydroxide cream buffered at a pH of 4.2, 9.21 c.c. of N/10 HCl in one-half hour, 10.34 c.c. in one hour, and 12.86 c.c. in two hours. One gram of the Alucol powder buffered at a pH of 4.2, 22.18 c.c. of N/10 HCl in one-half hour, 32.87 c.c. in one hour, and 34.97 c.c. in two hours.

The single doses of aluminum cream and Alucol actually used were 20 c.c. and 10 gm. respectively, such relatively large doses being chosen to obtain maximum effects, and to buffer theoretically from 250 to 350 c.c. of N/10 HCl in a two hour period, provided the acid and aluminum preparations were retained in the stomach, which of course, does not happen in vivo. In a one hour period these doses have the potentiality of buffering (pH 4.2) 206 c.c. and 328 c.c. of N/10 HCl respectively. The values for pH 4.2 only are given because we always titrated gastric samples to the yellow color of Töpfer's, an unmistakable end point.

To one group (5 dogs) 20 c.c. of aluminum cream, and to a second group (6 dogs), 10 gm. of Alucol were given four times a day, at 9 a.m. and at 1, 5, and 9 p.m. The dogs were fed at about 11 a.m., excepting on the days that test meals were fed. Medication was continued for more than four months.

Before starting the medication, an index of the secretion of the stomach was obtained (a) by feeding a test-meal and then aspirating a sample hourly for four hours, and also (b) by feeding the test meal three times per day and aspirating the stomach 4 hours after each meal, namely, at 1, 5, and 9 p.m. After starting medication the test meals and aspirations were performed about once weekly. The test meals were given with and without

*From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.
Submitted October 29, 1936.

TABLE I

Animals on continuous medication for sixteen weeks

Averages, daily	Before Medication Control		After Medication			
			First 9 weeks		Last 7 weeks	
	Free	Total	Free	Total	Free	Total
Aluminum Hydroxide Cream						
Plain Test Meals	.069	.220	.07	.23	.07	.20
Medicated Test Meals	.069	.220	.06	.19	.06	.21
Medicated Aspirations	.112	.240	.13	.28	.13	.25
Alucol						
Plain Test Meals	.077	.237	.11	.31	.11	.28
Medicated Test Meals	.077	.237	.05	.18	.07	.22
Medicated Aspirations	.131	.258	.14	.31	.16	.31

aluminum and the four hour aspirations were also made with and without the inclusion of aluminum.

The dose of aluminum was added to the meal; in the case of aluminum cream this was done throughout the period; in the case of Alucol, after several weeks the dogs would not eat their food with Alucol in it, so the Alucol was given by stomach tube, which did not influence the results because the dogs were habituated to taking the tube.

It should be pointed out that the aluminum preparations buffer alkali as well as acid. For this reason, although the values for free acid will be accurate, if much aluminum is present in the gastric sample the total acid value (phenolphthalein as an indicator) will be high. The nature of our results shows that the possible presence of aluminum in our gastric samples did not materially affect the total acid values. However, the greater significance is to be placed upon the free acid values. All titrations of gastric samples were made immediately.

RESULTS

The data are obviously very voluminous and cannot be given in entirety. The data, however, were sufficiently consistent to warrant the submission of averages for all dogs.

The averages on the five animals receiving 20 c.c. of aluminum cream 4 times daily for more than 4 months are shown in Table I. Similar data for Alucol are shown in Table I. The averaged acidity values of the non-medicated test meals before and after continued medication are shown as well as the medicated test meals before and after continued medication, and the four hour aspirations in which the meals had been medicated both before and after the continued period of medication. Such a scheme made it possible to determine the effect, if any, of long continued medication on the response of the stomach to non-medicated and medicated test meals. Any difference as much as ± 10% is significant.

It is obvious from an inspection of Table I that the administration of aluminum cream or Alucol in relatively large doses over a period of four months does not *decrease* the gastric secretory response to a test meal; no significant change occurred, the trend being toward a slight increase. In the case of Alucol and the plain test meals a definite increase occurred. This indicates that the stomach attempted to compensate for the buffering action of aluminum; and on the days that the non-medicated meal was given, the stomach continued to compensate as though aluminum had actually been added. This tendency to compensate is again shown in both the aluminum cream and Alucol data for the medicated aspirations, which were made 4 hours post-cibum, or at a time the stomach was practically free of food and medicament. As might be anticipated the acidity of the *medicated test meals* is reduced throughout the continued medication period, the reduction being greater for the first period of nine weeks than the latter seven weeks. This is not due to the stomach adapting itself to the aluminum after nine weeks or to the production of a gastritis, we believe, because when the average daily temperature meteorological curve is plotted alongside that of the gastric acidities, the decrease in acidities during the last seven weeks correlates with the average daily temperature, a fact that requires no discussion.

The attempt of the stomach to compensate for the effects of a neutralizing or buffering substance, of course, is a well-known phenomenon and occurs particularly in the case of alkalies (7). In the case of aluminum, however, the compensatory increase is not marked.

The effect of the addition of the aluminum preparations to a test meal. The same test meal employed in the former experiment was used and when medicated the same doses of the aluminum preparations given above were used. A gastric sample was obtained hourly for four hours.

Aluminum cream: Twenty-two animals were used. The non-medicated meal was fed 147 times and the medicated 137 times.

Alucol: The same experiment was conducted with Alucol on 28 animals. The non-medicated meal was fed 156 times and the medicated 117 times. The data are summarized in Table II.

First, it is to be noted that the data on the plain test meals in the two groups of animals (Table II) check remarkably, demonstrating the beauty of the averages of a large amount of data obtained under controlled conditions. The greater "buffering" effect obtained with Alucol in comparison to aluminum cream is due chiefly to the larger dose of Alucol administered, and we believe in part to the lower degree of solubility of Alucol. It is interesting that the ratio between the degree of lowering of free acidity at the 4 hour period in the case of aluminum cream and Alucol (0.028 : 0.038 = 74%) (Table II) is the same as the ratio between the "buffering" action of alumi-

TABLE II

Gastric acidities following test-meals (average values) expressed as per cent of HCl in 22 animals receiving Al cream

A	First Hour		Second Hour		Third Hour		Fourth Hour	
	Free	Total	Free	Total	Free	Total	Free	Total
Plain Test Meals	.018	.156	.044	.190	.087	.236	.125	.274
Medicated Test Meals	.014	.128	.034	.171	.073	.233	.097	.282
Reduction	.004	.028	.010	.019	.014	.003	.028	+.008
Per cent of reduction	22%	18%	23%	10%	16%	1%	22%	+3%

Gastric acidities following test meals (average values) expressed as per cent of HCl in 28 animals receiving Alucol

B	First Hour		Second Hour		Third Hour		Fourth Hour	
	Free	Total	Free	Total	Free	Total	Free	Total
Plain Test Meals	.017	.153	.045	.182	.082	.231	.113	.260
Medicated Test Meals	.009	.092	.027	.125	.055	.164	.073	.209
Reduction	.008	.061	.018	.057	.027	.067	.038	.051
Per cent of reduction	47%	48%	40%	31%	33%	29%	34%	20%

num cream and Alucol in the doses used at a 2 hour period (257.2 : 349.7 = 74%). This means that the differences observed are due chiefly to the differences in dosages of the two preparations.

Aspirations four hours after feeding, or at 1, 5, and 9 p.m. were made on both groups of animals, with and without the meals being medicated. In the aluminum cream group 82 "non-medicated" and 146 "medicated" daily aspiration tests were made; in the Alucol group 124 and 183, respectively. The animals did not receive more than 2 medicated sets of meals per week. These data because of their bulkiness will not be submitted. They agree essentially with the results shown under the fourth hour period in Table II. In fact, we made the aspirations to check the four hour data in Table II, and to observe if any accumulative effect of the aluminum might be observed to occur when given once or twice weekly at four hour periods during the day. A slight accumulative or compensatory effect was observed on the days the aluminum was given, which did not manifest itself when plain test meals were given.

HUMAN SUBJECTS

It is obvious that the ingestion of either of the two aluminum preparations will buffer gastric acid. However, we desired to perform a few experiments on several human subjects to ascertain how effective they might be.

Alcohol test meals: The normal gastric response of 6 graduate students, accustomed to the stomach tube, to 50 c.c. of 7% alcohol, after evacuating the gastric residuum, was determined. Then, the alcohol test was performed with Alucol (1.4 gm.) or Creamalin (4 c.c.), which was taken immediately after the alcohol. The curves (free acid only) of the average response of the group for two hours shows that the aluminum preparation reduced the free acidity for 45 minutes or longer. The variation in the response from subject to subject was rather marked, and the tendency to an increase in acidity after the aluminum had been evacuated, was characteristic of 4 of the 6 subjects.

Regular meals: The subjects (6 graduate students) in these experiments were instructed to choose a breakfast, a lunch and dinner and to ingest the same meals on three successive days, hourly samples of the gastric contents being withdrawn and titrated immediately. One day served as a control, and during the other two days either Alucol (1.4 gm.) or Creamalin (4 c.c.) were taken ½ hour after each meal and again at 10 a.m. and 3 and 8 p.m. The curves (free acid only) of the group averages show that the free acidity is reduced, but tends to rise to normal values an hour before the next meal. (It should be kept in mind that 4 c.c. of Creamalin and 1.4 gm. of Alucol buffer only about 40 c.c. of N/10 HCl in the test tube).

Similar experiments were performed in 10 subjects (students) except that the dose of the aluminum preparations was increased slightly and given hourly on the half hour for 14 hours. The subjects ate at 7 a.m., 12 noon, 6 p.m., and hourly at the half-hour they took 5 c.c. of aluminum cream (buffers 51.7 c.c. N/10 HCl in 1 hour and 46 c.c. in 0.5 hour) until 8:30 p.m. A gastric sample was removed on the hour and just before eating the noon and evening meal. A similar series of tests was made using 1.6 gm. of Alucol (buffers 52.6 c.c. N/10 HCl in 1 hour and 35.5 c.c. in 0.5 hour). Chart 1 shows the group averages and the results of subject Gr., whose free acid was reduced least of all. (The aluminum preparations tend to cause constipation in some normal subjects).

Effect of continuous and prolonged administration of aluminum hydroxide preparations on blood chlorides and plasma CO_2 combining power. The plasma CO_2 combining power and total blood chloride level were studied in eight dogs, four receiving 20 c.c. of aluminum cream and four 10 gm. of Alucol four times daily at 9 a.m., 1, 5, and 9 p.m. The plasma CO_2 combining power was found to be in the normal range for dogs, 46 and 56, average 49, volumes per cent. The blood chlorides expressed as sodium chloride ranged from 3.83 to 4.62, average 4.31 grams per liter which is slightly below or in the lower limits of the normal.

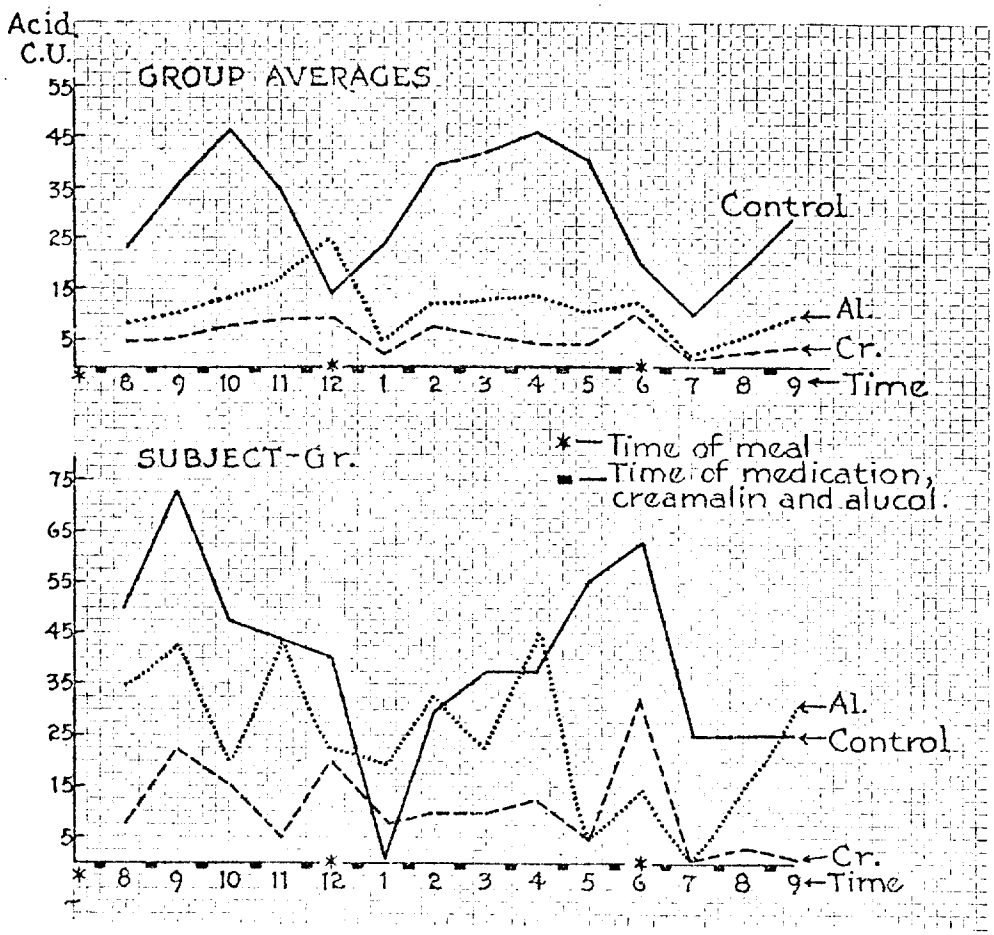


Chart 1

This confirms the findings of Einsel and others (2, 3) with the exception that our chloride values are somewhat lower, but our doses were relatively larger. The chloride values on patients receiving aluminum cream also fell in the lower limits of the normal range.

DISCUSSION

In view of the astringent action of aluminum hydroxide we rather anticipated a decrease in the gastric secretory response to a meal. No evidence showing that such occurs on the single or continued daily administration of the two preparations was obtained. The buffering action on the acidity of the gastric contents observed was of course to be expected and no significant difference in the two preparations in this regard was noted. It has been shown clearly by Boyd (7), using Pavlov pouch dogs, that alkalies such as sodium bicarbonate and calcium carbonate in ordinary doses given over a long period of time do not depress gastric secretion. Very large doses (3 gm. per kilo) depress, and on their withdrawal, a hypersecretion occurs. Small doses tend to augment secretion. No evidence was obtained indicating that the doses of the aluminum preparations used, given intermittently, stimulate secretion. However, the protracted administration of the preparations tended to augment secretion to non-medicated test meals given on days when medication was dithdrawn. This tendency of the stomach to compensate is analogous to that observed by Boyd in the case of alkalies, and in our experiment it cannot be ascribed to changes in acid base balance in the blood, since significant changes do not occur (2).

On the basis of our results pertaining to the amount of "buffering" of acid that occurred after the administration of the aluminum preparations, we doubt, as Einsel does, that the antacid properties of aluminum hydroxide preparations in the doses usually employed fully explain its reported effectiveness in "peptic" ulcer. In animals that have received the aluminum preparations for some time, one finds at autopsy the folds of mucosa in the stomach and duodenum covered with flaky curds, presumably precipitated mucin. Sometimes the duodenum is so coated that about two-thirds of its mucosa appears as if it had been covered with flour paste. This has been reported by numerous observers.

We chose the relatively large doses of the two aluminum preparations to administer to the dogs (a) because we doubted that one would desire to administer much larger daily doses to man, and never a larger per kilo dose, and (b) because we were interested in making observations on toxicity.

The question of the *toxicity of aluminum compounds* has been investigated and discussed pro and con at considerable length. The older literature has been reviewed by Smith (8). Unfortunately, much of the older work was not accompanied by histologic studies and the methods for the chemical analysis of tissue were not sufficiently sensitive and specific to render the results of value. Since 1929, however, more accurate methods (9-14a) for quantitating aluminum in biological materials have become available, the colorimetric method of Eveleth and Myers being the most suitable. It now appears established that rats may

be fed diets containing from 0.6 to 3.6 per cent aluminum chloride or other soluble aluminum salts for a year without producing histologic changes in their organs, affecting growth, producing anemia, or affecting reproduction (13-22). The only meager reports to the contrary (23) are incomplete. Aluminum compounds injected intravenously or subcutaneously in adequate doses are toxic, producing focal necrosis and swelling in the liver and kidney principally (11). So in the case of ingestion of aluminum, apart from the local irritating action of soluble aluminum compounds, the important question in regard to toxicity is how much aluminum is absorbed. Two studies (9a, 19) indicate that when soluble aluminum salts are perfused through an intestinal loop significant quantities are not absorbed, it being suggested that due to the alkaline reaction the colloidal hydroxide would be formed which one should hardly expect to be absorbed in appreciable quantities. When the more soluble compounds have been fed by mouth to rats and dogs in the amounts stated above, two investigations (13, 20) report no detectable absorption, four (14, 19, 11, 25) slight absorption, and two (23, 24) absorption in toxic amounts. As several investigators point out, the latter two investigations are open to criticism and could not be confirmed. Considering the results as a whole, it would appear that aluminum chloride, tartrate or sodium aluminum sulphate, when given in adequate doses orally, results in a slight increase in the aluminum content of tissues. Only figures ranging in the region of the maximum normal are obtained. All results show that the continued ingestion of aluminum does not lead to a continued or a cumulative deposition of aluminum in the liver and kidney. Aluminum subcutaneously causes anemia (26). But, in rats placed on a diet to cause a nutritional anemia, aluminum was found to exert no effect (27); in rats on a normal diet conflicting results have been reported (28, 18). The German Bureau of Health (29) fed dogs aluminum hydroxide in quantities corresponding with 1 gm. of aluminum oxide (Al_2O_3) daily for a period of twelve months. No change in the appetite or body weight occurred, and no histologic change in the organs of the animals was observed. The aluminum content of the organs was within the normal range. A similar dose was given to man without causing symptoms. Traces of aluminum were present in the urine, but a significant increase was not observed (12, 2, 3).

In our normal animals no significant changes in appetite and body weight have occurred with the relatively large doses of colloidal aluminum administered. However, we are interested in ascertaining the results of aluminum medication to Mann-Williamson dogs in which a tendency to hypersecretion of gastric juice exists along with a deficiency of alkaline pancreatic juice and bile to convert any aluminum chloride formed in the stomach to the insoluble hydroxide. To date our data for aluminum in the liver of such medicated dogs, receiving aluminum for from 3 to 8 months, have fallen in the normal range, except in one dog with a very fatty liver (aluminum, 0.14 mg. per gm. of dried liver). Underhill (11) found his highest hepatic content of aluminum in the liver of a man whose liver was fatty. Our complete data on this question will be reported when our results on Mann-Williamson dogs are completed.

CONCLUSIONS

1. When aluminum preparations (aluminum hydroxide cream and powdered colloid aluminum hydroxide powder) in relatively large daily doses, larger than recommended in the therapy of "peptic" ulcer in man, are administered for a period of 4 months to normal dogs, a decrease in the gastric secretory response to a meal does not result. The decrease in acidity reported to occur in ulcer patients on aluminum therapy must be due to other factors than the effect of the aluminum directly on the gastric secretory mechanism. Under prolonged aluminum administration the gastric secretory mechanism tends to compensate for the buffering action of aluminum, or to respond to other possible effects of aluminum, since we observed slightly higher acid values for the gastric contents when non-medicated test meals were used. The failure to observe this effect in human patients (2) may be due to the smaller doses employed clinically.

2. When aluminum preparations are administered with a meal in a relatively large dose once or twice weekly, no definite change in the gastric secretory response to a meal is observed. Temporary "buffering" of acidity is of course obtained.

3. The health of the animals was not impaired, as judged by the outward appearances, in spite of the relatively large doses of aluminum. The aluminum content of the liver of seven of eight dogs receiving the aluminum for a period of from three to eight months was within the normal range of variation. A review of the literature pertaining to the question of the toxicity of aluminum compounds is presented.

4. The effects of administering aluminum preparations, both hourly and six times a day, on the free acidity of the gastric contents in normal human subjects eating three meals a day are reported. As might be anticipated, the aluminum preparations buffer free acid and are more effective in this regard when administered more frequently.

REFERENCES

1. Crohn: *J. Lab. Clin. Med.*, 14:610, 1928-29.
2. Einsel, Adams and Myers: *Am. J. Dig. Dis. and Nutrit.*, 1:513, 1934-35.
3. Adams, Einsel and Myers: *Am. J. Dig. Dis. and Nutrit.*, 3:112, 1936.
4. Kogan: *Clinical Medicine*, 32:229, April, 1925.
   Gillermin: *Revue Med. d. l. Suisse Rom.*, 44:243, 1924.
5. Surmont and Cauvin: *L'Echo Medicine du Nord*, Oct. 10, 1925.
   Kreis: *Wien. Med. Woch.*, 81:140, 1931.
6. Woldman and Rowland: *Rev. of Gastro-enterology*, 3:27, 1936.
7. Boyd: *Am. J. Physiol.*, 71:455, 1924-25; 71:464, 1924-25.
8. Smith: Aluminum Compounds in Food. New York, 1928.
9. Eveleth and Myers: *J. B. C.*, 113:449, 467, 1936.
9a. Myers and Morrison: *J. B. C.*, 78:615, 1928.
10. Steel: *Am. J. Physiol.*, 22:94, 1911.
11. Underhill et al: *Am. J. Physiol.*, 90, 1, a series of articles, 1929.
12. Führer: *Biochem. Zeitschr.*, 265:169, 1933.
13. McCollum, Rask and Becker: *J. B. C.*, 77:753, 1928; *ibid.*, 85:779, 1930.
14. Myers and Mull: *J. B. C.*, 78:605, 1928.
14a. Lewis: *Biochem. J.*, 25:2162, 1931.
15. Rose and Catherwood: *J. Nutrit.*, 2:155, 1929-30.
16. Massatch: *Biochem. Zeitschr.*, 220:239, 1930.
17. Lyman and Scott: *Am. J. Hygiene*, 12:271, 1930.
18. Scott and Helz: *Am. J. Hygiene*, 16:865, 1932.
19. Mackenzie: *Biochem. J.*, 24:1433, 1930; 25:297, 1931; 26:883, 1932.
20. Tourtellotte and Rask: *Am. J. Hygiene*, 14:225, 1931.
21. Bertrand and Serbescu: *Compt. rend. Acad. Sci.*, 193:128, 1931.
22. Kraft: *Am. J. Hygiene*, 12:281, 1930.
23. Schaeffer, et al: *J. Hygiene*, 28:92, 1928.
    Ceriotti: *Rev. facult. cienc. quin.* (Univ. La Plat) 6:51, 1930.
24. Flinn and Inouye: *J. A. M. A.*, 90:1010, 1928.
25. Myers, Mull and Morrison: *J. B. C.*, 78:595, 1928.
26. Siebert and Wells: *Arch. Path.*, 8:230, 1929.
27. Mitchell and Miller: *J. B. C.*, 92:423, 1931.
28. Waltner and Waltner: *Arch. exp. Path. u. Pharm.*, 146:310, 1929.
29. Reichs: *Gesundheitsblatt*, 5:803, 1930.

J. Nutrition 19: 579-92 (1940)

A SPECTROCHEMICAL STUDY OF THE NORMAL RANGES OF CONCENTRATION OF CERTAIN TRACE METALS IN BIOLOGICAL MATERIALS [1]

ROBERT A. KEHOE, JACOB CHOLAK AND ROBERT V. STORY
Kettering Laboratory of Applied Physiology, University of Cincinnati, Ohio

ONE FIGURE

(Received for publication November 3, 1939)

It has come to be recognized that certain metals, once believed to be foreign and dangerous to living material, or to be accidental, at most, in their occurrence therein, are regular and normal constituents of the tissues and excretions of animals and men. Conclusions as to the effects of these metals on health and disease must be based, therefore, on quantitative information as to the limits of their normal concentration in biological materials, as well as to the ranges of concentration which may give rise to signs of deficiency on the one hand or of excess on the other. In the pursuit of such information considerations of convenience, specificity, and accuracy have led us to use quantitative spectrochemical methods for the estimation of a series of trace metals in various biological materials under a variety of conditions. Our methods for the determination of lead (Cholak, '35 a, '35 b), and bismuth (Cholak, '37), were recently improved and were extended so as to make possible the simultaneous determination of a number of metals in a single small sample (Cholak and Story, '38).

The data presented herein are concerned largely with the concentrations of manganese, lead, tin, aluminum, copper, and

[1] Presented before the Section of Biological Chemistry at the Ninety-eighth Meeting of the American Chemical Society at Boston, Mass., September 11–15, 1939.

silver which occurred in duplicates of the composite 24-hour food samples, in the excreta of apparently normal healthy human adults, and in the tissues of selected persons whose activities had given them no unusual or occupational exposure to these metals. A considerable volume of information on the lead content of individual food items, natural waters, and samples of soil from various portions of the earth's surface has accumulated since we recorded our earlier results and reviewed the work of other investigators (Kehoe et al., '33); this is presented herein. Reference to the numerous articles concerning the trace metals from other laboratories has of necessity been reserved for a separate review.

ANALYTICAL TECHNIQUE

Samples were prepared for analysis by procedures outlined previously (Cholak, '35 a, '35 b, '37; Cholak and Story, '38). Suitable quantities of solutions of the ashed materials, the inorganic salt compositions of which were made to conform to the standards by the method of excess (Cholak, '35 b, '37; Cholak and Story, '38), were placed in craters of purified graphite electrodes and their arc spectra were photographed while a five-step logarithmic sector was being rotated before the slit of the spectrograph (Cholak and Story, '38). The method of evaluating the spectral lines was similar to that of Strock ('36) except that opacities were substituted for densities in evaluating very weak lines (Cholak and Story, '38). The spectral region between 2600 A and 3500 A, in which the most persistent lines of a large number of the metals occur, was photographed. Although limited in application thus far to six metals, the method as applied to this region can be extended to include others, especially iron, thallium, cadmium and nickel. Zinc, on the other hand, cannot satisfactorily be dealt with simultaneously, since its most persistent lines at 3302 A and 3345 A lack sufficient sensitivity for the detection of the small amounts of zinc normally present and are also masked by the sodium line at 3302 A and the calcium line at 3345.5 A. The detection and determination of this metal are dependent, therefore, on spectrograms made by a special technique in the extreme ultraviolet region (Zn line at 2138.5 A), which lacks suitably persistent lines of other metals and for which specially sensitized plates must be used. In the case of manganese, included since our latest publication (Cholak and Story, '38), the line at 2801 A was employed in conjunction with the chromium line at 2835 A as internal standard. The latter metal was added to the mixed internal standard, which then consisted of 5 mg. of bismuth, 100 mg. of cobalt and 10 mg. of chromium per 100 ml. of solution (Cholak and Story, '38).

The quantitative sensitivity of the technique used in these analyses was further increased by concentrating the solutions of the ashed materials to a point beyond that of previous practice (Cholak and Story, '38). The volumes of the final solutions of tissues and whole blood were adjusted so that each cubic centimeter was equivalent to 2 gm. of the original material, while in the case of blood plasma each cubic centimeter was equivalent to 3 ml. of original plasma. Such procedures permitted the detection of 0.005 mg. of metal per 100 gm. of fresh tissue or blood, and 0.003 mg. of metal per 100 ml. of plasma.

RESULTS

The results obtained on normal human tissues are recorded in table 1. With the exception of the tissues of one male whose occupational history was carefully checked, those examined were from females who had never been employed in industry. This choice of material has limited the number of available samples and has prevented the establishment of statistically stable mean values for the concentration of metals in the various tissues, but the arithmetical means are expected to fall near the eventual stabile values.

Table 2 gives the data on the concentrations of the metals in the urine of several widely scattered groups of normal men, including thirty-four Frenchmen, thirty Mexican Indians and thirty Americans. The results with respect to lead on a group of thirteen Germans are also shown. The urine (24-hour speci-

TABLE 1

The concentration levels of trace metals in normal human tissues (Americans) (arithmetical mean values)

TISSUE	MILLIGRAMS OF METAL PER 100 GM. OF WET TISSUE					
	Mn	Pb	Sn	Al	Cu	Ag
Kidney	0.060	0.027	0.020	0.042	0.166	0.00
Heart	0.032	0.038	0.022	0.056	0.190	0.00
Brain	0.030	0.013	0.00	0.004	0.400	0.003
Liver	0.205	0.130	0.060	0.160	0.710	0.005
Spleen	0.022	0.030	0.022	0.130	0.085	0.00
Lung	0.022	0.028	0.045	5.94	0.110	0.004
Muscle	0.050 [1]	0.010	0.011	0.015	0.125	0.00
Long bone	0.300 [1]	1.88	0.089	0.500	1.190	0.00
Rib bone	0.170 [1]	0.470	0.050	0.240	0.410	0.01
Stomach	0.030 [1]	0.022	0.050	0.073	0.107	0.00
Intestines	0.035	0.023	0.016	0.087	0.110	0.002

[1] Results of single analyses.

TABLE 2

The concentrations of trace metals in normal urine expressed as the means, their probable errors, and their standard deviations

METAL	MILLIGRAMS OF METAL PER LITER OF URINE				
	Frenchmen	Mexicans	Americans	Germans	All groups
Mn		0.012±0.001 ±0.001	0.010±0. ? ±0. ?		0.01± ?
Pb	0.020±0.02 ±0.014	0.022±0.002 ±0.017	0.029±0.002 ±0.016	0.027±0.002 ±0.012	0.027±0.001 ±0.014
Sn	0.00±0.00 ±0.00	0.009±0.001 ±0.007	0.018±0.002 ±0.013		0.011±0.001 ±0.010
Al	0.114±0.006 ±0.048	0.054±0.004 ±0.031	0.052±0.003 ±0.022		0.078±0.002 ±0.032
Cu	0.036±0.003 ±0.026	0.039±0.003 ±0.024	0.028±0.002 ±0.019		0.034±0.002 ±0.024
Ag	0.00	0.00	0.00		0.00
No. of samples	34	30	30	13	94 [1]

[1] Total in case of lead 107, in case of manganese 60.

mens) of a healthy experimental subject was examined for 28 consecutive days and the results, expressed in milligrams of metal per liter of urine, are grouped in accordance with their frequencies of occurrence, in table 3, together with the calculated means, the probable errors of the means, and the standard deviations from the means.

Blood samples from thirty normal Americans and thirty normal Mexican Indians provided the results in table 4, in

TABLE 3

The concentrations of trace metals in successive daily urine samples of a normal adult American

MILLIGRAMS PER LITER	FREQUENCIES OF OCCURRENCE OF THE QUANTITIES INDICATED					
	Mn	Pb	Sn	Al	Cu	Ag
0.00 –0.009	28		7	1	2	28
0.010–0.019		1	17	1	5	
0.020–0.029		8	3	3	7	
0.030–0.039		14	1	5	3	
0.040–0.049		4		4	5	
0.050–0.059		1		4	1	
0.060–0.069				5	2	
0.070–0.079				1	2	
0.080–0.089				1		
0.090–0.099				2		
0.100–0.109						
0.110–0.119				1	1	
Number of samples	28	28	28	28	28	28
Mean	0.01	0.034	0.014	0.052	0.037	
Probable error	± ?	±0.001	±0.001	±0.003	±0.002	
Standard deviation	± ?	±0.008	±0.007	±0.025	±0.014	

which the mean concentrations of the metals in whole blood are shown. More detailed data obtained on twelve consecutive weekly samples of the blood of a normal subject appear in table 5. Blood samples of the American group were obtained in duplicate by dividing a 30 ml. sample of blood at the time of its withdrawal. One portion was used to determine the concentrations of the metals in the whole blood (table 4), while the other, after treatment with an anticoagulant (puri-

tied sodium citrate),[2] was centrifuged at once and the separated plasma was analyzed. The quantities of metals in the plasma of 100 gm. of whole blood were calculated in each case, and those in the formed elements were taken as the difference

TABLE 4

The concentrations of trace metals in normal blood

WHOLE BLOOD, MG. PER 100 GM.	Mn	Pb	Sn	Al	Cu	Ag
Mexicans:						
Mean	0.018	0.023	0.010	0.012	0.126	Trace
Probable error	±0.001	±0.0005	±0.0015	±0.001	±0.002	
Standard deviation	±0.010	±0.004	±0.012	±0.010	±0.018	
Americans:						
Mean	0.012	0.027	0.014	0.014	0.103	Trace
Probable error	±0.001	±0.0006	±0.0015	±0.002	±0.002	
Standard deviation	±0.006	±0.005	±0.012	±0.012	±0.013	
Whole group:						
Mean	0.015	0.025	0.012	0.013	0.114	Trace
Probable error	±0.001	±0.0004	±0.0009	±0.001	±0.002	
Standard deviation	±0.009	±0.005	±0.010	±0.011	±0.020	
DISTRIBUTION IN BLOOD (AMERICANS)[1]						
Whole blood:						
Mean	0.012	0.027	0.014	0.014	0.103	Trace
Probable error	±0.001	±0.0006	±0.0015	±0.002	±0.002	
Standard deviation	±0.006	±0.005	±0.012	±0.012	±0.013	
Plasma:						
Mean	0.004	0.0015	0.002	0.024	0.043	
Probable error	±0.0005	±0.0002	±0.0002	±0.002	±0.0015	
Standard deviation	±0.003	±0.0013	±0.0014	±0.012	±0.012	
Cells:						
Mean	0.008	0.024	0.011	0.003	0.059	
Probable error	±0.001	±0.0006	±0.001	±0.0003	±0.002	
Standard deviation	±0.006	±0.006	±0.008	±0.002	±0.016	

[1] Results based on 100 gm. of whole blood.

between plasma and whole blood. The calculated mean values for the whole blood, cells, and plasma of the group are listed in table 4. The histogram in figure 1 details the spread in the results obtained for the copper and lead content of the plasma.

[2] In order to check the possible precipitating effect of sodium citrate on the distribution of lead in the blood, a highly potent heparin preparation was used as the anticoagulant in a number of blood samples, with results similar to those in samples treated with sodium citrate.

TABLE 5

The concentration of trace metals in successive weekly blood samples of a normal adult American

MILLIGRAMS PER 100 GM.	FREQUENCIES OF OCCURRENCE OF THE QUANTITIES INDICATED						
	Mn	Pb	Sn	Al	Ag	MILLIGRAMS PER 100 GM.	Cu
0.000–0.0049			9	4	18	0.070–0.079	2
0.005–0.0099	5		3	5		0.080–0.089	
0.010–0.0149	4		4	3		0.090–0.099	4
0.015–0.0199	4		1	1		0.100–0.109	4
0.020–0.0249	4	1	1	1		0.110–0.119	2
0.025–0.0299		6		1		0.120–0.129	4
0.030–0.0349		1		1		0.130–0.139	2
0.035–0.0399	1	3		1			
0.040–0.0449				1			
Number of samples	18	11	18	18	18		18
Mean	0.016	0.030	0.008	0.015	0.00+		0.108
Probable error of mean	±0.001	±0.001	±0.001	±0.002	?		±0.003
Standard deviation	±0.008	±0.005	±0.006	±0.012	?		±0.018

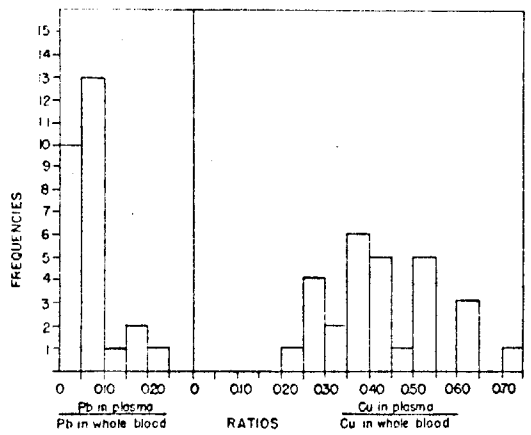


Fig. 1 Histogram showing the variation in the relationship between the plasma and the whole blood of normal human adults, with respect to their concentrations of lead and copper.

While the consecutive urine samples referred to in table 3 were being obtained, the corresponding feces and duplicate samples of the food and beverages taken by the subject were collected for analysis in 24-hour samples. The results are expressed in table 6 as mean daily intake and output.

Additional data concerning lead are given in tables 7 to 10.

TABLE 6

Comparison of the mean daily quantities of trace metals in successive samples of the feces and food of a normal adult American

	MILLIGRAMS PER 24 HOUR SAMPLE					
	Mn	Pb	Sn	Al	Cu	Ag
Food						
Mean	4.28	0.290	17.14	36.43	2.32	0.088
Probable error	±0.43	±0.027	± 1.30	± 7.90	±0.28	±0.010
Standard deviation	±3.38	±0.213	±10.17	±61.97	±2.21	±0.077
Feces						
Mean	3.69	0.320	22.88	41.92	1.96	0.058
Probable error	±0.30	±0.019	± 1.36	± 6.19	±0.18	±0.005
Standard deviation	±2.30	±0.141	±10.28	±46.80	±1.33	±0.035

DISCUSSION

The concentrations of these metals in normal tissues, blood and urine are seen to be low, and, excluding copper and silver, are of the same order of magnitude. The concentration of copper is seen to be severalfold that of the other metals in practically all tissues, while in the case of silver, which is irregular in occurrence, only minute amounts are encountered. The pattern of the distribution of these metals in the human organism is roughly similar in that their highest concentrations generally occur, in decreasing order of magnitude, in bones, liver, kidney and spleen. The most striking exceptions are the concentration of copper in the brain, which ranks next to that in the liver, and the concentration of aluminum in the lung, which is higher than that in any other tissue, largely, no doubt, because of the inhalation of dust. Since aluminum is the most widely distributed metal on the earth's surface, inhaled dusts, especially from streets, may be expected to be rich in aluminum. This omnipresence of aluminum may exert

a serious influence upon the accuracy of determinations of this metal, since dust may be carried on the shoes and clothing of workers into the most ideal laboratory, the air of which is filtered. Our consistently low and substantially uniform results indicate that such contamination was kept at a minimum. In this connection it is to be remembered that the samples of blood plasma were concentrated one-third more highly than were the whole blood samples, and thus they gave somewhat higher results than the latter, on account of the increased analytical accuracy obtained thereby and not because of contamination.

Manganese, lead, aluminum and copper were regularly present in all samples of human tissue and urine, while tin was found in about 80% and silver in only 10% of the samples. Although all metals but silver were frequently present in blood, there were decided differences in the distributions of these metals between the cells and plasma. Thus, manganese, lead and tin were located principally in the cells, aluminum in the plasma, and copper fairly evenly divided between the two, the cells usually containing slightly larger quantities.

All six metals occurred regularly in the feces and in composite food samples, and in concentrations considerably greater than those found in the tissues and the urine. In the case of each metal, the actual quantities found in the series of 24-hour samples of feces were of the same order of magnitude as those in the 24-hour samples of food, a fact which, in view of the low concentrations in the tissues and the urine, not only demonstrates that each of these metals is eliminated almost completely by the alimentary tract, but also suggests that they are but scantily absorbed by the alimentary tissues.

The detailed sources from which these metals are derived require further elucidation. It is well known that certain foods are high in manganese and copper, and that tin, aluminum, and silver in the dietary are derived to a large degree from the use of food containers, cooking utensils, and tableware, made of these metals. Only in the case of lead, however, are our data sufficiently numerous and varied to provide a

TABLE 7

The natural lead content of soil, water, and vegetation

MATERIAL	SOURCE	RANGE	NUMBER OF SAMPLES
		p.p.m.	
Soil (dry)	Northwestern U.S.A.	7.6 – 15.7	10[1]
Soil (dry)	State of Mexico, Mexico	0.70 – 8.0	5
Soil (dry)	Yucatan	0.80–25.0	38
Soil (dry)	Sarawac	1.20 – 3.0	3
Soil (dry)	River bottom in Sarawac	4.8	1
Tea leaves (dry)	Ceylon	0.02	1
Fruit of tree (wet)	Yucatan	0.15 – 0.30	2
Foliage (wet)	"	0.25 – 0.60	2
Bark (dry)	"	0.04 – 0.40	112
Root (dry)	"	0.05	1
Latex (wet)	"	0.04 – 0.08	36
Bark (dry)	Sarawac	0.04 – 0.25	6
Latex (wet)	"	0.02 – 0.04	4
Cocoa beans (dried husks)	Trinidad	0.40 – 1.60	14
Cocoa beans (dried nibs)	"	0.03 – 0.35	13
Peas (green)	State of Mexico	0.03	1
Peas (green pods)	" " "	0.05	1
Beans (green)	" " "	0.15 – 0.26	2
Beans (green pods)	" " "	0.04	1
Cherries (green)	" " "	0.05	1
Apples (green, unsprayed)	" " "	0.12	1
Pears (green, unsprayed)	" " "	0.18	1
Corn (green stalk)	" " "	0.05 – 0.11	3
Corn (green husk)	" " "	0.26	1
Corn (green on cob)	" " "	0.03 – 0.31	3
Wheat (green)	" " "	0.27	1
Radishes (green)	" " "	0.28	1
Sea water	Caribbean Sea	less than 0.02[2]	2
Sea water	Pacific Ocean	less than 0.02[2]	3
Sea water	Atlantic Ocean	0.003–0.005	2
River water	Sarawac	0.005	1
Pond water	"	0.004–0.02	3
Well water	Mexico	0.009	1
Stream water	"	0.009	1
Well water	U.S.A.	0.02 – 0.03	2

[1] Data of Jones and Hatch, Soil Science, vol. 44, p. 37 (1937).
[2] Limit of sensitivity of analytical method then in use.

fairly comprehensive picture of its origin in the diet. The wide distribution of lead is clearly indicated in tables 7 to 10. Its presence in the soil and in the vegetation growing therefrom, is shown in table 7. In our experience, as illustrated in these data, the natural lead content of vegetation rarely exceeds a small fraction of a part per million, but there is always some small quantity, whether the plant grows in the surface or is deeply rooted in the earth. There is a considerable variation among plants and in different parts of the same plant, with respect to lead concentration, but probably not to the extent indicated by the data, since certain of the samples could not

TABLE 8

The natural lead content of coral (Florida Keys)

LEAD IN PARTS PER MILLION	FREQUENCIES OF OCCURRENCE
0.00– 4.99	1
5.00– 9.99	7
10.00–14.99	11
15.00–19.99	4
20.00–24.99	1
25.00–29.99	
30.00	1
Total	25

TABLE 9

The lead content of bone (American). No occupational lead exposure

AGE IN YEARS	MILLIGRAMS Pb PER 100 GM. WET BONE	
	Rib	Femur
6	1.02	1.14
20?	1.11	
51	0.47	
64		0.80
70	0.39	3.59
75	0.60	2.89
95	0.56	1.36

be so handled as entirely to exclude the possibility of surface contamination with soil or atmospheric dust, although every effort was made to do so.

The occurrence of lead in natural waters is also indicated in table 7. The results on sea water bear a significant relationship to those obtained on a series of coral skeletons, as shown in table 8. The latter samples were obtained for us by R. L. Cary of Princeton University (whose assistance we gratefully acknowledge), from the Tortugas Laboratory of the Carnegie Institution, with such precautions in packing as would prevent surface contamination. These analyses were made because of speculation as to the possibility of the development of substantially lead-free soils on a coral island. Since it is apparent

that there is a selective absorption of lead by the coral skeleton, it is scarcely to be expected that a soil founded upon such material would support the growth of lead-free plants and animals.

TABLE 10

The lead content of various foods and drinks

ARTICLE	RANGE	NUMBER OF SAMPLES
	p.p.m.	
Wheat bread	0.02–0.16	8
Bran flakes	0.14–0.15	2
Crackers and pretzels	0.24–0.25	2
Spaghetti (prepared for eating)	0.06–0.21	2
Corn (dry)	0.24	1
Cornstarch	0.75–1.83	4
Corn syrup	0.21–0.49	2
Cocoa (20 brands)	0.4–11.5	25
Tea (dried leaves) Chinese	43.2	1
Cabbage	0.10–0.24	4
Fruits (raw and cooked)	traces–1.00	16
Beef liver (fresh)	0.29–0.40	2
Meat (cooked)	traces–0.63	9
Meat (ground, cooked)	0.15–0.18	3
Sausages (cooked)	0.16–1.60	4
Eggs (raw and cooked)	traces–0.12	6
Coffee (prepared for use)	0.01–0.03	2
Milk	0.02–0.04	3
Beer	0.01–0.09	21
Grape juice	0.04–0.40	7
Wine (domestic and imported)	0.05–1.51	10
Water	0.02–0.05	10
Water	0.37–0.92 [1]	3

[1] Obtained from building in which water was standing unused in pipes for some days.

As shown by the standard deviation of the mean value (table 6) the freely chosen daily diet of a normal healthy adult shows considerable variation in its lead content. It is not to be assumed that all such lead is of natural origin, although the results on commonly available foods and beverages, as listed in table 10, show that such is the origin of a considerable proportion of it. As we have pointed out elsewhere (Kehoe et al., '33), the increment of lead content above that which is a

natural constituent is due to contamination from a great variety of sources.

The essential equivalence of the mean daily intake and output of lead, as previously referred to in commenting on the results for the entire group of six metals, is worthy of special note in connection with the data of table 9, which fail to reveal any relationship between age and lead concentration in the skeletal tissues. These data are obviously too few to give direct and definite evidence against an indefinitely progressive accumulation of lead in the body in response to the customary regular intake of lead over a prolonged period, but they are presented because of the care taken against the inclusion of abnormal material among the samples.

SUMMARY

A quantitative spectrographic method of high sensitivity and precision has been employed for the simultaneous determination of lead, manganese, tin, aluminum, copper and silver in normal biological material.

1. Lead, manganese, copper and aluminum were present in all materials examined; tin was present in about 80% and silver in 10 to 20% of the samples.

2. The mean concentrations of these metals in a liter of normal urine has been found to be below 0.01 for manganese and 0.078, 0.034, 0.027, 0.011, and 0.00 mg. respectively for aluminum, copper, lead, tin and silver.

3. The mean concentrations of the metals in 100 gm. of normal whole blood are 0.114, 0.025, 0.015, 0.013, 0.012, and 0.00+ mg. respectively for copper, lead, manganese, aluminum, tin and silver.

4. Practically all of the manganese, lead and tin is contained in the formed elements of the blood; aluminum is found almost entirely in the plasma, while copper is almost evenly divided between the two, the formed elements usually containing a slightly higher concentration.

5. The concentrations of these metals in consecutive daily or weekly samples of urine and blood from the same individual are not constant but vary from sample to sample.

6. The daily output of this group of metals in the feces is practically equivalent to their daily intake in the diet.

7. The wide distribution of lead is indicated by data obtained on a large number of natural materials.

LITERATURE CITED

CHOLAK, J. 1935 a The quantitative spectrographic determination of lead in urine. J. Am. Chem. Soc., vol. 57, p. 104.

———— 1935 b Quantitative spectrographic determination of lead in biological material. Ind. Eng. Chem., Anal. Ed., vol. 7, p. 287.

———— 1937 Spectrographic analysis of biological material. II. Bismuth. Ind. Eng. Chem., Anal. Ed., vol. 9, p. 26.

CHOLAK, J., AND R. V. STORY 1938 Spectrographic analysis of biological material. III. Lead, tin, aluminum, copper and silver. Ind. Eng. Chem., Anal. Ed., vol. 10, p. 619.

KEHOE, R. A., F. THAMAN AND J. CHOLAK 1933 On the normal absorption and excretion of lead. I. Lead absorption and excretion in primitive life. II. Lead absorption and excretion in modern American life. III. The sources of normal lead absorption. IV. Lead absorption and excretion in infants and children. J. Ind. Hyg., vol. 15, pp. 257–305.

STROCK, L. W. 1936 Spectrum Analysis with the Carbon Arc Cathode Layer. Adam Hilger, London.

The Effect of Aluminum Hydroxide on the Acid-Base Balance and on Renal Function

By

JOSEPH B. KIRSNER, M.D.*
CHICAGO, ILLINOIS

ALUMINUM hydroxide was first introduced in the treatment of peptic ulcer in 1922 by Roch (1). Guillermin (2) and later Crohn (3) found that it reduced the emptying time of the stomach, lowered gastric acidity, and caused no harmful side effects. Subsequently a large number of clinical reports (4) have uniformly emphasized the value of colloidal aluminum hydroxide and this preparation is now widely used in the therapy of gastro-duodenal ulcer and various forms of gastritis. One of the most important qualities attributed to aluminum hydroxide is its inability to produce alkalosis even though large quantities are administered. This claim has been supported by the clinical observations of Rutherford

*Department of Medicine, University of Chicago.
Submitted October 22, 1940.

TABLE I

	Case	Sex Age	Total Amt. Aluminum Hydroxide	No. Days	Avg. Daily Amt. (cc.)	ACID BASE BALANCE					
						Cl (100-110 mM/L)	CO_2 (22-30 mM/L)	pH (7.35-7.45)	Blood Urea Nitrogen (9-15 mgm. %)	Urea Clearance	Day of Treatment
1.	H.H. 228115	F 35	312 cc.	6	52	96.2 96.9	25.6 26.1	7.45 7.45	12.3 ...	86% ...	4th 6th
2.	P.B. 230768	M 38	454 cc.	11	41	98.4 102.9	28.6 27.0	7.45 7.47	17.1 12.4	84% 180	4th 11th
3.	G.F. 239655	M 40	528 cc.	22	24	103.4 108.2	30.3 29.7	7.38 7.42	... ...	... ...	1st 22nd
4.	J.S. 203840	M 29	956 cc.	21	46	102.3 98.4 97.8 98.6 102.8	29.3 28.2 31.9 30.7 31.6	7.45 7.47 7.49 7.48 7.46	11.0 9.2 8.0 8.2	84% ... 140 140 121	4th 7th 12th 18th 21st
5.	N.W.S. 141313	M 29	1564 cc.	23	68	... 101.7 ... 100.7	... 25.9 ... 28.3	... 7.48 ... 7.48	13.4 12.6 ...	100% 97 ...	1½ yrs. previously 1st 7th 23rd
6.	F.McC. 235528	M 43	2230 cc.	30	75	101.0 94.0 94.2 96.3 98.0	29.9 31.6 30.0 33.7 31.3	7.45 7.48 7.48 7.48 7.48	16.9 14.0 ... ... 8.6	59% 104 81	1st 3rd 6th 10th 30th
7.	E.F.C. 232945	M 67	2255 cc.	29	78	97.3 102.9 100.4	29.5 27.5 29.8	7.47 7.48 7.47	14.7 8.9 ...	71% 65 ...	1 mo. previously 4th 29th
8.	A.K. 237776	M 20	2920 cc.	73	40	99.4 103.3 102.8	29.6 28.7 28.9	7.46 7.49 7.47	... ...	... ...	14th 43rd 73rd
9.	L.J. 238664	M 35	3024 cc.	84	36	100.0 103.6 100.5 101.0	29.4 24.9 28.4 29.7	7.42 7.43 7.43 7.42	... ...	... ...	1st 14th 42nd 84th

Am. J. Digestive Diseases 1
Vol. VIII - No. 5, May, 1941 pp 160-163

and Emery (5), Eads (6), Woldman and Poland (7), Brown and Dolkart (8) and others. However, there have been relatively few reported studies of the effect of aluminum hydroxide on the acid-base balance. In 1934, Einsel, Adams and Myers (9) reported that in five cases this antacid did not elevate the blood CO_2 content nor significantly alter the total base and blood chlorides. Bennett and Gill (10) likewise observed

TABLE I (CONTINUED)

	Case	Sex Age	Total Amt. Aluminum Hydroxide	No. Days	Avg. Daily Amt. (cc.)	ACID BASE BALANCE Cl	CO_2	pH	Blood Urea Nitrogen	Urea Clearance	Day of Treatment
10.	H.E. 188969	M 46	3268 cc.	31	105	100.2	27.1	7.48	...	...	2 days previously
						100.9	29.6	7.51			2nd
						95.9	29.9	7.49	14.9	41.1%	6th
						98.2	30.5	7.47			9th
						100.4	30.1	7.47	10.7	52.5	15th
						...	...	...	9.9	54.2	22nd
						104.0	29.1	7.46	10.4	40.4	28th
						100.0	27.0	7.44	...	...	31st
11.	A.K. 207457	M 48	3290 cc.	45	73	98.0	27.3	7.50	...	...	1st
						...	...	...	27.0	22.0%	2nd
						...	...	...	19.6	27.0	3rd
						101.7	28.5	7.48	18.5	32.0	4th
						105.0	27.1	7.43	13.5	37.0	7th
						103.6	24.9	7.46	8.9	30.0	11th
						106.1	25.4	7.48	11.9	34.0	18th
						104.8	27.4	7.43	12.5	42.0	29th
						...	...	...	13.1	63.0	36th
						101.1	29.6	7.47	13.8	58.0	45th
12.	A.R. 234911	M 26	3534 cc.	107	33	102.6	23.7	7.49	11.1	98%	3 days previously
						99.4	27.9	7.49	12.0	144	4th
						103.9	25.6	7.48	7.7	131	13th
						99.6	27.5	7.46	...	...	19th
						100.8	28.6	7.47	...		46th
						...	...	...	9.7	125	66th
						103.5	24.3	7.50	...	...	107th
13.	A.E. 56366	F 36	3640 cc.	78	47	102.8	26.7	7.47	...	...	1st
						102.2	29.9	7.46	...	...	21st
						...	30.3	7.47	...	...	49th
						105.7	28.2	7.43	...	...	78th
14.	A.R. 182058	M 37	3965 cc.	93	42	95.2	29.0	7.48	...	...	4th
						105.7	24.6	7.45	...		8th
						104.8	26.2	7.45	...	...	12th
						104.0	27.7	7.43	...	...	93rd
15.	J.McD. 226545	M 48	4400 cc.	220	20	101.8	28.5	7.43	...	...	176th
						105.5	28.0	7.48	...	...	220th
16.	P.R. 234794	M 38	6448 cc.	144	45	96.8	28.9	7.46	...	...	1st
						98.7	29.5	7.46	...	...	8th
						98.3	32.8	7.44	...	...	15th
						98.6	30.1	7.43	...	...	29th
						...	30.9	...	...	...	50th
						95.9	32.0	7.43	15.3	54%	71st
						...	...	...	15.9	61	116th
						96.4	30.4	7.50	...	...	144th
17.	F.G. 19177	M 58	6600 cc.	174	38	100.5	29.4	7.48	...	...	5 days previously
						100.5	28.5	7.43			3rd
						102.1	28.5	7.43	14.0	57%	8th
						100.8	20.3	7.43	...	...	13th
						102.6	29.1	7.42			42nd
						...	...	...	19.5	62	77th
						103.3	28.0	7.43	16.9	72	122nd
						107.0	29.7	7.43	...	...	133rd
						101.0	28.7	7.48	...	...	174th
18.	V.J. 119460	M 40	8412 cc.	207	41	103.4	29.2	7.42	...	...	184th
						104.2	30.1	7.45	...	...	207th
19.	L.A. 223689	M 29	10,648 cc.	193	54	104.5	29.2	7.43	16.8	120%	Control
						101.5	30.9	7.43	...	...	29th
						103.5	32.2	7.44	...	...	43rd
						102.6	31.7	7.48	...	...	79th
						104.1	30.5	7.50	...	...	96th
						...	...	...	11.3	81	160th
						100.2	33.9	7.47	...	...	193rd
20.	M.G. 171869	F 45	10,976 cc.	261	42	104.9	26.8	7.38	...	...	116th
						106.4	28.2	7.43	...	...	261st

TABLE I (CONTINUED)

	Case	Sex Age	Total Amt. Aluminum Hydroxide	No. Days	Avg. Daily Amt. (cc.)	ACID BASE BALANCE Cl	CO_2	pH	Blood Urea Nitrogen	Urea Clearance	Day of Treatment
21.	M.G. 229503	M 19	11,462 cc.	116	99	98.6	28.8	7.49	11.9	88%	1st
						99.6	27.7	7.46	...	...	6th
						100.8	29.8	7.49	8.6	111	12th
						97.6	30.7	7.48	8.4	82	20th
						101.0	29.3	7.48	...	...	40th
						100.9	28.6	7.47	11.3	148	59th
						100.9	31.9	7.46	14.5	100	116th
22.	H.M. 92827	M 47	18,984 cc.	241	79	...	27.5	7.55	20.2	69%	6 years previously Alkalosis just prior to use of aluminum hydroxide
						...	...	...	16.7	63	
						79.2	45.1	7.59	54.5	13	
						99.7	28.7	7.53	32.2	21	
						102.4	26.3	7.45	21.2	29	
						101.0	28.5	7.46	16.9	36	4th
						103.8	25.2	7.43	18.4	38	7th
						100.2	25.4	7.48	16.0	44	11th
						104.4	27.7	7.46	18.7	59	28th
						....	...	...	22.6	46	42nd
						100.8	....	....		...	56th
						101.4	29.0	7.44	20.0	71	91st
						102.6	28.2	7.46	26.1	69	161st
						99.2	28.7	7.44	21.0	82	241st
23.	N.F. 227265	M 67	27,905 cc.	71	393	....	....	....	14.3	68	21st
						101.2	30.8	7.46		...	27th
						....	....	....	12.4	82	28th
						105.0	27.4	7.41	...	...	71st

a normal electrolyte equilibrium during aluminum hydroxide therapy; their series included one patient with a urea clearance of only 19 per cent of normal. Recently, McIntosh and Sullivan (11) noted, in 14 cases, no tendency for this preparation to elevate the blood CO_2 content or the non-protein nitrogen.

In view of the lack of detailed information on this most important subject, the present investigation was undertaken to determine more accurately and in detail the influence of aluminum hydroxide on the acid base balance and on renal function. For this purpose, varying quantities of aluminum hydroxide* were administered to 23 patients with peptic ulcer.

Studies of the acid-base balance were made at variable intervals during the course of therapy, as indicated in the table. These determinations included (a) serum chloride (Normal—100-110 millimols per liter, mM/L), (b) serum CO_2 (normal 22-30 mM/L), (c) serum pH (normal—7.35-7.45). In addition, the blood urea nitrogen (normal 9-15 mgm. per cent) and the urea clearance test of renal function were determined in 14 of the 23 patients. The urea clearance is expressed as per cent of average normal after correction for individual surface area (lower limit of normal—75 per cent).

The results are shown in the table. It will be noted that the acid-base balance remained within normal limits in every instance despite the ingestion of massive amounts of aluminum hydroxide. Case 22 (H. M.), for example, received 18,984 cc. in 241 days, an average of more than 78 cc. daily for 8 months. Case 23 (N. F.) received the largest quantity of aluminum hydroxide hitherto reported, 27,905 cc. in 71 days, an average of 393 cc. daily, without harmful effect on the electrolyte balance or on renal function. The longest period of observation was 261 days (Case 20).

The blood urea nitrogen and urea clearance remained normal from the onset of treatment throughout the period of observation in 11 patients. The three individuals with lowered renal function are of particular interest.

Case 22 (N. M.) was the first in which aluminum hydroxide was administered immediately following recovery from a severe alkalosis. It will be noted that, although the urea clearance had decreased to 13 per cent of average normal, it improved steadily over a period of 241 days of aluminum hydroxide therapy and, at the present time, is within normal limits.

Case 10 (H. E.) was similarly treated several days after recovery from a marked alkalosis. During this time the serum chloride had decreased to 66.3 mM/L, the serum CO_2 had risen to 51.8 mM/L, and the pH to 7.67; the blood urea nitrogen was 75 mgm. per cent while the urea clearance had decreased to 7.5 per cent of average normal. Despite the administration of more than 100 cc. aluminum hydroxide daily for a month, the blood chemistry remained normal and the urea clearance steadily improved. Case 11 (A. K.) is similar and illustrates again that the use of large quantities of aluminum hydroxide does not interfere with the gradual return to normal of a markedly depressed renal function. The urea clearance in this patient is now 90 per cent of normal.

SUMMARY

The effect of aluminum hydroxide on the acid base balance was determined in 23 patients with peptic ulcer. Three patients received more than 100 cc. daily for as long as 31 to 116 days; one patient received 393 cc. daily for 71 days. The period of observation in several cases exceeded 7 to 8 months. The electrolyte

*The aluminum hydroxide used was the product, Amphojel, generously supplied by the John Wyeth and Brother Company, Philadelphia, Pa.

balance was normal in every instance. The blood urea nitrogen and urea clearance tests for renal function were determined in 14 patients of the group. Renal efficiency was not decreased in any patient in whom had been normal. The use of aluminum hydroxide in three patients with a marked reduction in kidney function following alkalosis, was followed by the maintenance of a normal acid base balance and by a gradual improvement in the urea clearance.

CONCLUSIONS

1. Aluminum hydroxide, even in massive amounts, does not disturb the acid-base balance.
2. Aluminum hydroxide administered in large amounts over periods as long as seven to eight months does not impair renal function.
3. Aluminum hydroxide may be administered with complete safety to individuals with a marked reduction in renal efficiency.

REFERENCES

1. Roch, M.: *XVI Congres Francais de Médicine*, Paris, p. 101, 1922.
2. Guillermin, R.: Hyperchlorhydri et hydrate d'aluminumo colloidal. *Rev. med. de la Suisse Rom.*, 44:243, 1924.
3. Crohn, B. B.: The Clinical Use of Colloidal Aluminum Hydroxide as a Gastric Antacid. *J. Lab. and Clin. Med.*, 14:610, 1929.
4. Kreis, L.: Kausale Radikalheilung der Hyperacidital der konservative Behandlung. *Wien. med. Wochschr.*, 81:140, 1931.
   Einsel, I. H. and Rowland, V. C.: Aluminum Hydroxide Treatment of Peptic Ulcer. *Ohio State Med. J.*, 28:173, 1932.
   Woldman, E. E. and Rowland, V. C.: Continuous Acid Absorption by Aluminum Hydroxide Drip in the Treatment of Peptic Ulcer. *Rev. Gastro-Enterol.*, 3:27, 1936. A New Technique for the Continuous Control of Acidity in Peptic Ulcer by the Aluminum Hydroxide Drip. *Am. J. Dig. Dis. and Nutrit.*, 2:733, 1936.
   Jones, Clement R.: Colloidal Aluminum Hydroxide in the Treatment of Peptic Ulcer. *Am. J. Dig. Dis. and Nutrit.*, 4:99, 1937.
   Ivy, A. C., Terry, L., Fauley, G. B. and Bradley, W. B.: The Effect of Administration of Aluminum Preparations on the Secretory Activity and Gastric Acidity of the Normal Stomach. *Am. J. Dig. Dis. and Nutrit.*, 3:879, 1937.
   Emery, E. S., Jr. and Rutherford, R. B.: Studies on the Use of Aluminum Hydroxide Gel in the Treatment of Peptic Ulcer. *Am. J. Dig. Dis.*, 5:486, 1938.
   Quigley, J. P., Einsel, I. H. and Merchan, I.: Some Effects Produced in the Normal Stomach by the Ingestion of Moderate or Massive Quantities of Aluminum Hydroxide Gel. *J. Lab. and Clin. Med.*, 24:485, 1939.
   Kunstler, M. B.: Gastritis: Its Treatment with Colloidal Cream of Aluminum Hydroxide Lavage. *Medical Record*, 149:268, 1939.
   Kyger, E. R., Hashinger, E. H. and Wilkeny, E. W.: Treatment of Peptic Ulcer with Colloidal Aluminum Hydroxide. *Am. J. Dig. Dis.*, 6:363, 1939.
   Steigmann, F.: Colloidal Aluminum Hydroxide Continuous Drip in the Treatment of Large Gastric Ulcers: The Therapeutic and Diagnostic Value of This Method. *Ill. Med. J.*, 76:443, 1939.
   Komarov, S. A. and Krueger, Luise: The Effect of Aluminum Hydroxide Gel on Gastric Secretion in the Dog. *Am. J. Dig. Dis.*, 7:170, 1940.
5. Rutherford, R. B. and Emery, E. S., Jr.: Clinical Effect of Colloidal Aluminum Hydroxide on Patients with Peptic Ulcer. *N. E. J. Med.*, 220:407, 1939.
6. Eads, J. T.: Clinical Results from the Continuous Intra-Gastric Drip. *Am. J. Dig. Dis.*, 7:32, 1940.
7. Woldman, E. E. and Poland, C. G.: The Value of Colloidal Aluminum Hydroxide in the Treatment of Peptic Ulcer. *Am. J. Med. Sci.*, 198:155, 1939.
8. Brown, C. F. G. and Dolkart, R. E.: An Evaluation of the Therapy of Peptic Ulcer. *J. A. M. A.*, 113:278, 1939.
9. Einsel, I. H., Adams, W. L. and Myers, V. C.: Aluminum Hydroxide in the Treatment of Peptic Ulcer. *Am. J. Dig. Dis. and Nutrit.*, 1:513, 1934.
10. Bennett, T. I. and Gill, A. M.: Colloidal Aluminum Hydroxide in the Treatment of Peptic Ulcer. *Lancet*, 1:500, 1939.
11. McIntosh, J. F. and Sutherland, C. G.: The Use of Colloidal Aluminum Hydroxide in the Treatment of Peptic Ulcer. *Canad. M. A. J.*, 42:140, 1940.

THE EFFECT OF CALCIUM CARBONATE, ALUMINUM PHOSPHATE, AND ALUMINUM HYDROXIDE ON MINERAL EXCRETION IN MAN[1]

By JOSEPH B. KIRSNER

(From the Frank Billings Medical Clinic, Department of Medicine, University of Chicago, Chicago)

(Received for publication July 3, 1942)

INTRODUCTION

In order to elucidate further the mechanism of the alkalosis observed during the alkali treatment of peptic ulcer, a detailed study of mineral excretion following the administration of relatively non-absorbable antacids seemed desirable. Hence, an investigation of the influence of calcium carbonate on mineral excretion in man was undertaken, and its action compared with the effects of aluminum hydroxide and aluminum phosphate, two non-absorbable compounds.

METHOD OF STUDY

Studies were carried out in 2 adult men and one female with peptic ulcer, in all of whom previous observations had indicated renal function to be satisfactory. A special metabolic ward and nursing staff were utilized. The patients were maintained on special ulcer diets (Tables I and II), without change, during the entire experiment. Chloride-free distilled water was used for drinking purposes. The daily fluid intake averaged approximately 3000 cc. The daily urinary output varied from 1200 to 3000 cc. There was no significant change in body weight during the entire experiment. The only medication permitted, aside from the substances studied, was the occasional administration of cascara to facilitate daily bowel activity,[2] and the use of mild sedatives for sleep. An interval of 4 to 6 days was allowed for adjustment to the diet, prior to the control observation. Mineral excretion was studied over periods of 4 days duration each. Ten grains of carmine were given at the onset of each period and repeated 96 hours later; the feces obtained between the carmine markers was collected for analysis. The 4-day collection was mixed thoroughly; accurately weighed 2.0 gram samples were taken for the various determinations. All analyses were done in duplicate or triplicate.

The chemical studies of the feces were carried out as follows:

The calcium content was determined by first boiling the sample with concentrated nitric acid and potassium permanganate. After cooling, the mixture was diluted with distilled water to 100 (or 200) cc. volume and adjusted to the proper pH (4.6 to 5.2), using Brom Cresol green as indicator. The procedure then followed was the same employed for blood serum (1). (Calculation: $\frac{\text{Titer}}{2} \times 10$ equals mgm. Ca per 1 gram feces.)

TABLE I

Composition of diet employed in case A. K.
(Daily values)

	Special diet	Standard values for sedentary adult female
Carbohydrates	198	
Protein	72	
Fat	104	
Calories	2016	
Chloride (mgm.)	2974	
Sodium (mgm.)	2029	
Potassium (mgm.)	3052	
Calcium (mgm.)	1159	800
Phosphate (mgm.)	1342	1320
Iron (mgm.)	10.5	12
Vitamin A (I.U.)	9570	5000
Thiamin (mgm.)	1.014	1.2
Riboflavin (mgm.)	2.51	1.8
Ascorbic Acid (mgm.)	78	70
Vitamin D (I.U.)	38	

TABLE II

Composition of diet employed in cases C. D. and W. G.
(Daily values)

	Special diet	Standard values 70 kgm. adult male
Carbohydrate	266	
Protein	58	
Fat	163	
Calories	2763	
Chloride (mgm.)	3047	
Sodium (mgm.)	2014	
Calcium (mgm.)	1140	800
Phosphate (mgm.)	1300	1320
Iron (mgm.)	7	12
Vitamin A (I.U.)	6200	5000
Thiamin (mgm.)	1.0	1.5
Riboflavin (mgm.)	1.2 to 2.4	2.2
Ascorbic acid (mgm.)	5 to 30	75
Vitamin D (I.U.)	160 to 385	

[1] This work was supported in part by a grant from John Wyeth and Bro., Inc., Philadelphia, Pa.

[2] There was no instance of diarrhea.

J. Clin. Invest. 22(1): 47-52 (1943)

For determining the chloride content, 5.0 cc. of a 0.1 N solution of silver nitrate were added to 2.0 gram samples of feces and the mixtures allowed to stand for several hours. Digestion then was carried out as with the calcium analysis. The mixtures were cooled, 5 per cent ferric alum added as indicator, and then titrated with a 0.1 N solution of potassium thiocyanate. (Calculation: $\frac{5 - \text{titer} \times 3.5}{2}$ equals mgm. chloride ion in 1.0 gram feces.)

The phosphate in the feces was determined in Case C. D. by wet ashing of the samples with nitric and sulphuric acids and, when necessary, superoxal. The mixtures then were cooled, adjusted to known volume with distilled water, and the phosphate content measured by the Fiske-Subbarow method (2) as adapted for the Evelyn photoelectric colorimeter. In Cases A. K. and W. G., the samples of feces were ashed in a muffle furnace and the analyses then carried out as above.

In Case A. K., the sodium content of the feces was measured by the following procedure: 20 cc. of ferric sulphate and 3 cc. of concentrated sulphuric acid were added to 10 gram samples. The mixtures were dried in an oven and then ashed in a muffle furnace. The ash was taken up in 10 cc. of distilled water and 3 cc. aliquots were analyzed for sodium by the method employed for blood (3).

The potassium content of the feces in Case A. K. was determined as follows: 2.0 gram samples of feces were dried in an oven and then ashed in a muffle furnace. The ash was taken up in 10 cc. of distilled water and 1.0 cc. aliquots were analyzed by the procedure used for blood (4).

Twenty-four hour collections of urine were obtained throughout the study. The urine was voided directly into a bottle containing toluene as a preservative and a layer of mineral oil to prevent the escape of CO_2 (5). The urine was kept in an icebox and analyzed at the end of each collection for the following: (*a*) pH, using the Beckman pH meter, (*b*) chloride (6), (*c*) calcium, (*d*) phosphate, and (*e*) amino-nitrogen plus ammonium salts (7).[3]

The sodium content of the urine in Case A. K. was measured by the same technique employed for blood, modified as to the volume used; ferric salt was added in amounts sufficient to prevent interference by phosphates. The urinary potassium was determined as follows: 2.0 cc. samples of urine were ashed in a muffle furnace and the ash taken up in 10 cc. of distilled water; 2.0 cc. aliquots then were concentrated and analyzed by the method employed for blood.

Simultaneous studies were made of the serum electrolytes. Venous blood drawn under oil was utilized for the following analyses: chloride, CO_2 (8), pH (9), calcium, and phosphorus. Oxalated blood was used for the measurement of the blood urea nitrogen (10). The following additional data were obtained in Case A. K.: cell volume, serum water (11), blood sodium, potassium, and total base (12), and the urea clearance (13).

After control values had been established, the effects of calcium carbonate, aluminum phosphate, and aluminum hydroxide were studied in individual periods. A 4 to 5 day interval for adjustment to the added medication was allowed prior to the analyses. Calcium carbonate was administered in 2.0 gram amounts 10 times daily: 80 grams (400 m.eq.) were thus given in a 4-day period, containing 32 grams of calcium ion. One hundred and five cc. of aluminum phosphate were administered daily in divided doses; a total of 420 cc. (35 m.eq. HPO_4) in a 4-day period containing, by analysis, 386 mgm. of chloride, 4368 mgm. of phosphate, and 2625 mgm. of aluminum (14). One hundred and five cc. of aluminum hydroxide were given daily in divided doses; a total of 420 cc. in a 4-day period containing 386 mgm. of chloride and 4716 mgm. of aluminum. Complete mineral balances were not obtained since no attempt was made to measure the loss in the insensible perspiration or sweat. This loss presumably was constant, however, through the various periods in each case, since fairly constant conditions were maintained. The results are considered exclusively in relation to the individual control values.

RESULTS

The complete data are recorded in Tables III and IV.

DISCUSSION

(*1*) *Calcium carbonate*

The ingestion of 400 m.eq. daily, as calcium carbonate, was roughly accounted for by increase in total outgo, almost entirely in the stools. The increase in excretion in the urine was, for the patient A. K., only 7.3 m.eq., and for the other 2 patients, C. D. and W. G., 13.9 m.eq. and 14.1 m.eq., respectively. As was to be expected with the large addition to calcium excretion in the stools (15), there was an increase of HPO_4 in the feces. For A. K. and W. G., the outgo in the stools was about double the fore-period values. These increments were, however, somewhat more than offset by a decrease in the removal of HPO_4 in the urine. The increase in calcium and decrease in HPO_4 excretion in the urine together markedly reduced the requirement for ammonium production as noted in Table III. Other compounds of acid-base excretion, however, are related to the change in ammonium production. For the patient A. K., a small increase in Na and decrease in Cl, and a considerable reduction of K, were found. The re-

[3] Referred to in the text as "ammonia" for the purpose of simplicity.

TABLE III

Effect of calcium carbonate, aluminum phosphate, and aluminum hydroxide on mineral excretion in man

Subject	Periods of study (4-day)	Urine							Feces					Total outgo				
		pH	NH_4	Na	K	Ca	HPO_4*	Cl	Na	K	Ca	HPO_4*	Cl	Na	K	Ca	HPO_4*	Cl
		m.eq. per 24 hours							*m.eq. per 24 hours*					*m.eq. per 24 hours*				
A. K. Unit No. 148736 Female Age 63 Gastric ulcer	Fore-period, I	6.16	34.3	54.5		11.6	31.2	72.8	1.3		30.6	10.5	2.0	55.8		42.2	41.7	74.8
	Fore-period, II	6.27	35.6	59.0	63.3	12.6	30.5	82.3	0.9	6.6	41.3	10.9	2.0	59.9	69.9	53.8	41.4	84.3
	Average	6.21	35.0	55.7	63.3	12.1	30.9	77.6	1.1	6.6	36.0	10.7	2.0	57.9	69.9	48.0	41.6	79.6
	$CaCO_3$, I (400 m.eq.)	6.56	20.6	58.8	49.1	20.5	12.1	71.0	1.7	11.9	356.0	24.9	4.1	60.5	61.0	376.5	37.0	75.1
	$CaCO_3$, II	6.75	21.0	69.3	52.1	20.2	10.4	81.2	1.9	12.1	394.0	24.8	3.9	71.2	64.2	414.2	35.2	85.1
	$CaCO_3$, III	6.63	26.2	49.5	50.2	17.5	12.7	73.2	0.7	12.0	378.0	20.5	4.0	50.2	62.2	395.5	33.2	77.2
	Average	6.65	22.6	59.2	50.5	19.4	11.7	74.8	1.4	12.0	376.0	23.8	4.0	60.6	62.5	395.4	35.1	79.1
	Change	+ 0.44	− 12.4	+ 3.5	− 12.8	+ 7.3	− 19.2	− 2.8	+ 0.3	+ 5.4	+ 340.0	+ 12.7	+ 2.0	+ 2.7	− 7.4	+ 347.4	− 6.5	− 0.5
	After-period	5.92	36.6	70.5	60.6	8.6	30.4	85.2	2.0	10.6	38.8	9.7	2.7	72.5	71.2	47.4	40.1	87.9
C. D. Unit No. 255381 Male Age 37 Duodenal ulcer	Fore-period	5.76	67.5			12.0	26.7	66.6			54.3	14.4	2.5			66.3	41.4	69.1
	$CaCO_3$ (400 m.eq.)	6.24	37.2			25.9	8.9	23.7?			484.0	17.8	2.9			509.9	16.7	26.6
	Change	+ 0.48	− 30.4			+ 13.9	− 17.8	− 42.9			+ 429.7	+ 3.4	+ 0.4			+ 443.6	− 24.7	− 42.5
	Aluminum phosphate, I (35 m.eq. HPO_4)	5.42	88.4			9.5	24.5	87.1			46.3	41.1	4.3			55.8	65.6	91.4
	Aluminum phosphate, II (35 m.eq. HPO_4)	5.43	77.2			8.9	29.6	76.0			51.4	42.7	4.6			60.3	72.3	80.6
	Average	5.42	82.8			9.2	27.1	81.6			48.9	41.9	4.5			58.1	69.1	86.0
	Change	− 0.34	+ 15.3			− 2.8	+ 0.4	+ 17.0			− 5.4	+ 27.5	+ 2.0			− 8.2	+ 27.7	+ 16.9
	Aluminum hydroxide	5.93	46.8			12.1	7.3	60.5			51.9	33.2	3.9			64.0	40.5	64.4
	Change	+ 0.17	− 20.7			+ 0.1	− 20.7	− 6.1			− 2.4	+ 18.8	+ 1.4			− 2.3	− 0.9	− 4.7

TABLE III—*Continued*

Subject	Periods of study (4-day)	Urine							Feces					Total outgo				
		pH	NH_4	Na	K	Ca	HPO_4*	Cl	Na	K	Ca	HPO_4*	Cl	Na	K	Ca	HPO_4*	Cl
		m.eq. per 24 hours							*m.eq. per 24 hours*					*m.eq. per 24 hours*				
W. G. Unit No. 144032 Male Age 51 Gastric ulcer	Fore-period	5.16	73.0			6.3	24.5	75.0			54.3	17.5	2.9			60.6	42.0	77.9
	$CaCO_3$ (400 m.eq.)	6.06	38.9			20.4	8.2	57.7			520.0	30.3	5.3			540.4	38.5	63.0
	Change	+ 0.9	− 34.1			+ 14.1	− 16.3	− 17.3			+ 465.7	+ 12.8	+ 2.4			+ 479.8	− 3.5	− 14.9
	Aluminum phosphate (35 m.eq.)	5.12	86.7			5.5	24.5	80.5			40.2	38.7	8.7			45.7	63.2	85.2
	Change	− 0.04	+ 13.7			− 0.8	± 0.0	+ 5.5			− 14.1	+ 21.2	+ 1.8			− 14.9	+ 21.2	+ 7.3
	Aluminum hydroxide	5.59	43.3			5.5	8.1	73.3			43.8	25.0	4.8			49.3	33.1	78.1
	Change	+ 0.47	− 29.7			− 0.8	− 16.4	− 1.7			− 10.5	+ 7.5	+ 0.9			+ 11.3	− 8.9	+ 0.2

* HPO_4 is here taken as univalent for the reason that at the usual pH of urine its base equivalence is approximately 1.0.
The values for "change" are derived by comparison with the fore-period values.

TABLE IV

Effect of calcium carbonate, aluminum phosphate, and aluminum hydroxide on various constituents of the blood plasma

Subject	Periods of study (4-day)	Cl	CO_2	pH	P	Ca	K	Na	Total base	BuN	Urea clearance	Cell volume	Serum water
		mM./L.			*mM./L.*		*m. eq./L.*			*mgm. per cent*	*per cent average normal*	*per cent*	*grams per cent*
A. K. Unit No. 148736 Female Age 63 Gastric ulcer	Fore-period I	101.9	30.9	7.43	1.26	4.9	5.30	143.0	155.3	15.4	66	40.4	91.1
	Fore-period II	102.2	31.8	7.47	1.29	4.7	5.20	144.3	153.3	14.6	81	37.0	90.97
	Calcium carbonate I	100.7	31.7	7.50	1.35	5.75	5.20	147.9	160.0	17.0	44	41.0	90.42
	Calcium carbonate II	100.5	31.9	7.44	1.19	5.35	5.00	145.2	158.5	17.8	65	41.5	90.68
	Calcium carbonate III	103.1	29.7	7.47	1.22	5.1	5.30	145.0	156.1	15.5	65	37.5	91.14
	After-period	100.2	30.9	7.43	1.13	5.1	5.30	143.8		13.2	76	37.2	91.06
C. D. Unit No. 255381 Male Age 37 Duodenal ulcer	Fore-period	101.7	29.6	7.46	1.32	5.05				9.4			
	Calcium carbonate	99.6	29.6	7.46	0.97	5.15				13.5			
	Aluminum phosphate I	103.4	28.4	7.48	1.0	5.7				12.0			
	Aluminum phosphate II	100.5	29.8	7.46	1.22	5.5				10.0			
	Aluminum hydroxide	102.4	28.5	7.47	1.13	5.25				15.4			
W. G. Unit No. 144032 Male Age 51 Gastric ulcer	Fore-period	96.7	28.6	7.43	1.42	4.65				14.2			
	Calcium carbonate	95.6	29.9	7.43	1.35	5.1				13.8			
	Aluminum phosphate	106.9	28.2	7.42	1.40	4.75				16.3			
	Aluminum hydroxide	101.6	27.4	7.41	1.40	5.05				14.0			

maining components, Mg, SO_4, and organic acids were not measured. The accuracy of ammonium adjustment is shown by the slight extent of change in urine pH. For the patient W. G., a considerable, and for C. D., a much larger, reduction of chloride in the urine was found. Excretion of this anion in the urine is known to fluctuate widely even in the presence of a constant intake (16, 17).

The changes in mineral excretion induced by calcium carbonate occurred within 24 hours after the addition of the alkali to the regimen. Mineral excretion returned to original levels almost equally soon after the discontinuation of calcium carbonate therapy.

The use of calcium carbonate did not alter the electrolyte constitution of the blood plasma (Table IV).

(2) *Aluminum phosphate*

The ingestion of 35 m.eq. HPO_4 as aluminum phosphate caused a roughly equivalent increase in the stools and had no appreciable effect on HPO_4 output in the urine. Calcium excretion in the stools was to a slight extent reduced. There was no appreciable change in the calcium output in the urine. Some increase in chloride and, also, ammonium excretion in the urine was found. A relationship of these changes to phosphate ingestion was not apparent. No alterations were noted in the electrolyte components of the blood plasma.

(3) *Aluminum hydroxide*

Aluminum hydroxide caused a considerable increase in HPO_4 excretion in the stools, as demon-

strated by the data from the two periods of study. This finding is in agreement with the results obtained by other workers (18). A roughly equivalent decrease of the excretion of HPO_4 in the urine was found and, along with it, the to be expected reduction of ammonium. For patient C. D., this was exactly equivalent to the decrease of HPO_4. The total outgo of HPO_4 was not increased. Aluminum hydroxide did not alter the electrolytes of the blood plasma, thus confirming previous observations (19).

CONCLUSION

The ingestion of calcium carbonate, aluminum phosphate, or aluminum hydroxide, in the quantities used in the treatment of peptic ulcer, places no appreciable burden on the processes of acid-base metabolism. The necessary adjustments of acid-base excretion are relatively small and are accomplished with a remarkable precision.

The electrolyte constitution of the blood plasma is not disturbed.

The author grgtefully acknowledges the generous aid given by Dr. James L. Gamble in the preparation of the paper and the valuable technical assistance of Miss Jacqueline S. Front.

BIBLIOGRAPHY

1. Clarke, E. P., and Collip, J. C., A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., 1925, 63, 461.
2. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phophorus. J. Biol. Chem., 1925, 66, 375. Applied to Evelyn Photoelectric Colorimeter, *cf.* Bulletin No. 460, Rubicon Co., Philadelphia, Pa.
3. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for the determination of sodium in biological material. J. Biol. Chem., 1931, 93, 171.
4. Shohl, A. T., and Bennett, H. B., A micro method for the determination of potassium as iodoplatinate. J. Biol. Chem., 1928, 78, 643. Modified by Eichelberger.
5. Marshall, E. K., Jr., The effect of loss of carbon dioxide on the hydrogen-ion concentration of the urine. J. Biol. Chem., 1922, 51, 3.
6. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. J. Biol. Chem., 1928, 79, 221.
7. Henriques, V., and Sörensen, S. P. L., Ueber die quantitative Bestimmung der Aminosäuren, Polypeptide und der Hippursäure im Harne durch Formoltitration. Ztschrf. f. Physiol. Chem., 1909, 63, 27.
8. Van Slyke, D. D., and Neil, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem., 1924, 61, 523.
9. Hastings, A. B., Sendroy, J., Jr., and Robson, W., The colorimetric determination of the pH of the urine. J. Biol. Chem., 1925, 65, 381.
10. Van Slyke, D. D., Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. J. Biol. Chem., 1927, 73, 695.
11. Hastings, A. B., and Eichelberger, L., The exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solutions. J. Biol. Chem., 1937, 117, 73.
12. Stadie, W. C., and Ross, E. C., A micro method for the determination of base in blood, serum and other biological material. J. Biol. Chem., 1925, 65, 735.
13. Van Slyke, D. D., and others, Observations on the courses of different types of Bright's disease and on resultant changes in renal anatomy. Medicine, 1930, 9, 257.
14. Front, J., and Kirsner, J. B., An improved method for the determination of aluminum in biological material using the Evelyn photoelectric colorimeter. J. Lab. and Clin. Med., 1942, 27, 1598.
15. a. Forbes, E. B., and Keith, M. H., A review of the literature of phosphorus compounds in animal metabolism. Ohio Agricultural Exp. Station, Technical Series Bulletin No. 5, Wooster, Ohio, 1914.
    b. Telfer, S. V., Studies on calcium and phosphorus metabolism. I. The excretion of calcium and phosphorus. Quart. J. Med., 1922, 16, 45.
16. Gamble, J. L., Chemical Anatomy, Physiology and Pathology of Extracellular Fluid. A Lecture Syllabus. Dept. Pediatrics, Harvard Medical School, Boston, 1939.
17. Atchley, D. W., Loeb, R. F., *et al.*, On diabetic acidosis. J. Clin. Invest., 1933, 12, 297.
18. a. Fauley, G. B., Ivy, A. C., Terry L., and Bradley, W. B., An attempt to prevent post operative jejunal ulcer by aluminum hydroxide therapy. Am. J. Digest. Dis., 1939, 5, 792.
    b. Fauley, G. B., Freeman, S., and Ivy, A. C., Aluminum phosphate in the therapy of peptic ulcer. Arch. Int. Med., 1941, 67, 563.
    c. Freeman, S., and Freeman, W. M. C., Phosphorus retention in children with chronic renal insufficiency. Am. J. Dis. Child., 1941, 61, 981.
19. Kirsner, J. B., The effect of aluminum hydroxide on the acid-base balance and on renal function. Am. J. Digest. Dis., 1941, 8, 160.

carried down deep until it grazes the bone and close enough together to hold the soft tissue and eliminate dead spaces, then a dressing is put on the same as you would use for any kind of incision, and the patient is put to bed and from then on all you do is to extend him the ordinary courtesies of invalidism.

I have had seven hospital deaths in connection with this work, but none of them have been attributable to the accident or to the operation. One died with cancer of the uterus during the year, one died with a kidney stone, one died with multiple arthritis, two died with pneumonia. These people can sit up in bed if they so desire and they do not have to be in a horizontal position. Within 10 days most of them can bend the leg to an angle of 45°.

I bring this to show you in the hope that you will adopt this method because it is so simple, yet the most efficient that has been proposed.

We use this method of treatment in all fractures of the shaft of the femur anywhere and we have now one case in the shaft of the femur which had three years and ten months duration of non-union. We obtained a union in four weeks.

What I want to draw your attention to more than anything elese is that it is a method by which you can relieve old people and you can use it with the young if you want to.

I am submitting an illustration of Dr. R. W. Waldrop's case which is the best example I have seen of the durability and reliability of this plan of treatment. I wish to acknowledge my obligation to Dr. Waldrop for his permission to use this case.

REPORT OF A CASE OF ALUM POISONING

LOUIS LEVY, M. D.
NEW ORLEANS

White and Wilcox has this to say about the internal use of alum: "In large doses it is emetic acting directly on the stomach and in larger doses, still, it is irritant and purgative. Most, if not all, is passed by the feces, probably in medicinal doses it has no more remote effect on the tissues. Nervous system: Given to animals in large doses it produces paresis, loss of sensation, forced movements, drowsiness, and death from respiratory paralysis." With this description of the toxicosis of alum, one can readily see that alum is a poisonous drug. Only a few years ago the use of alum was discontinued in baking powder on account of its toxic properties

Since this case had been confused with gall bladder and gastric conditions, and the correct diagnosis cleared the picture, it should be of more than usual interest.

CASE REPORT

On May 31, 1932, Mr. C. W. J. called at my office complaining of severe pains after meals, accompanied with nausea, which lasted most of the time. Loss of weight. "Felt that if he did not get relief he was going to die." History of gastric lavage and many gall bladder drainages with no results. Also treated for hysteria.

General examination: Male about 36 years of age. Height 6 feet 1 inch. Weight 115 pounds. Emaciated, muscles tapered to strings. Glands, negative; chest, negative; lungs, negative; Abdomen, slight regidity in epigastrum; liver, normal, reflexes, normal. Clinically, with the exception of pain in epigastrum, this patient was negative. Urine, negative in ordinary chemical analysis and microscopical examination. The conclusion of roentgen-ray report was spastic colon. (Drs. Fortier and Gately).

No diagnosis was made and after a few examinations patient was requested to report occasionally after being placed on a bland diet, he having refused to return to a gastroenterologist.

Some days later his brother came in to see me saying that a neighbor had told him: "That the reason Mr. S. could not get well, was that his wife was putting alum in his food with the idea of killing him and leaving no evidence."

Without letting the patient know of this information, I advised the brother to allow him to eat his usual meal, this meal was prepared by his wife, and to bring him to Hotel Dieu for removal of same by stomach tube. He reported promptly after his dinner and the meal was removed. This meal was sent to the laboratories of Dr. Couret and Hauser with the history of the case. The report of the chemist was: "Alum in easily discernable quantity." The urine was also sent, and showed alum in the form of potash alum.

The patient was advised to change his diet and abode without the knowledge of the findings. He did as directed, and with the cooking of a different source he showed gradual improvement. No medication was given, and under the new alum free diet he gained twenty pounds in three months. He is free of any signs or symptoms, as we know now, of alum poisoning.

M. & S. J. 86:620 (1934)

EFFECTS OF THE INGESTION OF TARTRATE OR SODIUM ALUMINUM SULFATE BAKING POWDERS UPON GROWTH, REPRODUCTION AND KIDNEY STRUCTURE IN THE RAT.*

By

J. F. LYMAN AND ERNEST SCOTT.

(Received for publication May 1, 1930.)

The absorption of aluminum by the animal body after the ingestion of its salts and the effects on digestion and health of aluminum baking powders have been studied by many experimenters. The results which are more or less conflicting, have been collected and summarized by E. E. Smith (1). McCollum, Rask and Becker (2) have reported that food containing 600 parts per million of aluminum, supplied by sodium aluminum sulfate-calcium phosphate baking powder or by aluminum chloride had no effect on growth, reproduction or well being in white rats to which it had been fed as an exclusive diet. Using a spectrographic method, they obtained no evidence that aluminum was absorbed into the blood or tissues after long continued feeding.

Underhill and Peterman (3) have recently developed a new colorimetric method for the quantitative determination of small amounts of aluminum in tissues and have applied their method to the study of the occurrence of aluminum in food products, its absorption from the digestive tract, its distribution in the body and its elimination. They find that aluminum is generally distributed in food-stuffs, that small quantities of it are absorbed but that it is readily excreted into the bile and urine and also through the intestinal wall. They had no evidence of toxic effects when baking powders that contained aluminum were added to the food. Injected subcutaneously, aluminum chloride or aluminum sulfate were toxic, the lethal dose of the salts varying from 5 to 8 grams per kilo of animal.

Myers and Mull (4) administered per os 2 milligrams of aluminum per day per rat as potassium aluminum sulfate and found no evidence of injury as judged by growth and reproduction records, even though the experiment was continued through the fourth generation. The

* From the Department of Agricultural Chemistry and the Department of Pathology, The Ohio State University, Columbus, Ohio.

Am. J. Hyg. 12: 271-288 (1930)

aluminum content of the tissues showed only a very slight increase after the aluminum feeding.

The doses of aluminum in Myer's experiments were relatively large, corresponding to from two to three times that which the Referee Board (5) considered a large amount. McCollum and his associates fed a food mixture containing a fixed percentage of aluminum so that in his experiments the daily intake would vary directly with the total food intake. The amount administered by McCollum, however, would be in the neighborhood of two to three times as much as Myers and Mull used.

A series of papers by Schaeffer, Fontes, Breton, Oberling, and Thivolle (6) describe experiments with men, dogs, fowls, rats and mice which show an unfavorable effect of aluminum-containing baking powders on growth, reproduction and health. The doses of baking powder used by Schaeffer and his associates were very much greater than those employed by McCollum, Rask, and Becker. Schaeffer used as the main article of diet, a bread with the following formula: flour 230 grams, baking powder 16, salt 2, lard 28, milk 190. In certain cases the baking powder was increased to two or to three times this quantity. Assuming that the milk contained 10 per cent solids (skim milk), 16 grams of baking powder would be contained in 295 grams of bread solids, therefore, the aluminum content (baking powder contained 2.1 per cent aluminum) would be approximately .105 per cent in the lowest dosage used and .21 and .30 per cent in the higher doses. Assuming a food consumption of 10 grams per rat per day, the daily dose of aluminum would vary from 10.5 milligrams to 30 milligrams, amounts 5 to 15 times as large as those used by Myers and about 2 to 5 times as large as those used by McCollum. The detrimental effects noted by Schaeffer and his associates include, delayed gastric digestion, delayed growth, gastro-intestinal irritation as shown by diarrhea and lesions in the gastric and intestinal mucosa and reproduction abnormalities as shown by a decreased number of offspring and histological changes in the ovaries.

The doses used by Schaeffer were so large, however, that 5, 10 or 15 per cent of the total food solids consisted of baking powder residues.

We have fed a colony of white rats for two years on dietaries containing relatively small to large doses of sodium aluminum sulfate—calcium acid phosphate baking powder (S.A.S. powder) or cream of tartar baking powder with results in general agreement with those obtained by McCollum, Rask and Becker and by Myers and Mull.

Methods.

Young white rats about four weeks of age and weighing 30 to 50 grams, obtained from the Albino Supply, Inc., Philadelphia, were divided into seven groups of approximately 24 rats in each group. Some replacements were made as original animals died. In some cases the rats used for replacement weighed 90 to 120 grams when placed upon the experimental diet. The baking powders were prepared for feeding as follows: The baking powder was moistened with water, 100 grams S.A.S. powder with 50 cc. or 100 grams tartrate powder with 40 cc., and the wet mixture heated in an oven regulated at 102° for one hour. The reaction mixtures were then air dried and ground to pass a 40 mesh sieve. These residues were mixed with the food as described below, fresh food being mixed at intervals of one to two weeks.

The basal ration contained:

Wheat meal	6 parts
Poultry meat scraps	1 part
Dried skim milk	1 part
Lard or butter	2 parts
Salt	0.1 part

Group 1 was fed the control diet without addition of baking powder residue.

Group 2 received the control diet plus 1 gram of S.A.S. baking powder residue to 223 grams of food mixture, corresponding to 1 gram of baking powder to 212 grams of food.

Group 3 received the control diet plus one gram of S.A.S. baking powder residue to 56 grams of food mixture, corresponding to 1 gram of baking powder to 53.5 grams of food.

Group 4 received the control diet plus 1 gram of S.A.S. baking powder residue to 669 grams of food corresponding to 1 gram of baking powder to 636 grams of food.

Group 5 received the control diet plus 1 gram of tartrate baking powder residue to 116 grams of food, corresponding to 1 gram of baking powder to 100 grams of food.

Group 6 received the control diet plus 1 gram of tartrate baking powder residue to 29 grams of food, corresponding to 1 gram of baking powder to 25 grams of food.

Group 7 received the control diet plus 1 gram of tartrate baking powder residue to 340 grams of food, corresponding to 1 gram of baking powder to 292 grams of food.

In addition to the above diets, which were fed ad libitum, cod-

liver-oil was fed separately several times a week at the rate of about one half grams per week per rat. Fresh cabbage or other green stuff was fed occasionally.

The baking powders used were well-known commercial products and were purchased from a local market. Calumet brand represented the S.A.S. phosphate type and Royal the tartrate type.

The diets were so mixed that the amounts of baking powder ranged from nothing in the control, to an amount equivalent to that which would be contained, on the calorie basis, in biscuits made according to the recipes on the baking powder containers, with two intermediate rations, one corresponding to a diet in which baking powder was used in a small part of the diet only, and one corresponding to a diet in which foods prepared with baking powders were prominent but not exclusive. Our largest doses of S.A.S. baking powder would be about 4 milligrams aluminum per rat per day, i.e. about double the doses used by Myers and Mull and somewhat smaller than those used by McCollum.

The rats were individually caged in wire cages provided with screen bottoms of sufficiently large mesh so that the feces dropped through out of reach of the rat. Cages were cleaned and animals weighed weekly. Care was taken to keep the feed cups and water bottles sanitary. The reproduction records of the first generation animals do not represent the maximum reproductive capacity of the animals since males and females associated only at intervals and the cages were not well adapted for breeding. Third, and succeeding generations on the S.A.S. ration were kept in larger cages, however, in pairs and their reproduction rates indicate about the maximum of which the animals were capable. The amount of S.A.S. residue fed to the third and succeeding generations was double the largest amount given the first generation group, i.e. 1 gram of S.A.S. residue to 28 grams of food, was used for the succeeding generations. Thus the daily intake of aluminum in the third, fourth, fifth and sixth generation rats would be in the neighborhood of 8 milligrams per day, the amount of baking powder residue in this food being nearly 4 per cent.

At intervals of about two months representative animals were killed by a blow on the head, blood collected by severing the jugular vein and samples of tissues taken for histological examination. Altogether 40 animals were killed for these tests. The blood samples were analyzed for non-protein nitrogen as a test for a possible deranged kidney function.

The rates of growth, maximum size, and the number of deaths

occurring within 5 months after starting the experimental diets are shown in the following table 1.

TABLE 1.

Diet	Av. time to gain from 60 to 160 gr., days	Maximum variations, days	Av. max. wt. of rats living 5 months or longer	No. dying under 5 months after starting diet	Total no. of rats	No. animals used to calculate rate of gain
			Males.			
Control	47	35 to 56	260	2	13	8
Control + S.A.S. 1 to 223	47	42 to 56	275	2	10	8
Control + S.A.S. 1 to 56	42	28 to 59	263	1	14	11
Control + S.A.S. 1 to 669	46½	28 to 59	263	0	8	7
Control + tartrate 1 to 116	47½	38 to 59	261	3	10	6
Control + tartrate 1 to 29	44	28 to 59	277	5	14	8
Control + tartrate 1 to 340	57	49 to 70	284	3	8	3
			Females.			
	To gain from 50 to 130 grms.					
Control	40	31 to 56	193	2	9	4
Control + S.A.S. 1 to 223	37	33 to 45	180	7	14	6
Control + S.A.S. 1 to 56	39	28 to 59	207	2	17	9
Control + S.A.S. 1 to 669	37	16 to 66	190	3	13	5
Control + tartrate 1 to 116	40	31 to 52	212	4	14	7
Control + tartrate 1 to 29	52	31 to 63	197	4	14	7
Control + tartrate 1 to 340	54	38 to 84	192	2	10	5

The rates of growth seem to be accelerated slightly by the S.A.S. calcium acid phosphate and slightly retarded by the tartrate residues, although the results are not definite. The number of animals used is too small to permit the use of the statistical method for analysis of

the data. In one group only 3 animals and in 2 others only 5 animals were suitable for the measurement of growth rates. This was due to the fact that in some cases the animals were too large at the start of the experiment so that growth over the selected interval could not be measured.

One male on the lowest S.A.S. diet, one male on the control diet and one female on the high tartrate diet were not considered in calculating the average growth rates because in all three cases growth over the interval chosen was very slow, 109, 105 and 119 days being required to gain from 60 to 160 grams for the males and 50 to 130 grams for the female respectively. Since these slow growth rates were so different from those of the other individuals of the groups it is probable that they are due to causes not resident in the diets consumed.

Reproduction and rearing of young: The method of handling the first generation rats in separate cages with only occasional association between the sexes did not result in a large number of offspring. No young were born on the control diet, seven second generation litters were born on the S.A.S.-calcium acid phosphate diets, and there were eight second generation litters on the tartrate diets. The fact that no young were born on the control diet probably has no significance in terms of dietary effects. Since on the S.A.S. ration there was one litter to 6.3 females, and on the tartrate ration one litter to 4.8 females, while on the control diet there were no litters to 9 females, it is evident that the majority of females did not reproduce. The second generation animals were kept in individual cages like those used for the first generation; but the third and following generations were kept in pairs in large cages. The reproduction record for two pairs of rats, representing the third generation and fed from weaning and thereafter S.A.S.-calcium acid phosphate baking powder residue at the rate of 1 gram of residue to 28 grams of food is given in table 2 below.

These records are considered remarkably good. From March 2, 1929, to October 25, 1929, pair no. 1 produced 79 young of which 52 were successfully weaned. Pair no. 2 from March 5, 1929, to October 25, 1929, produced 53 young of which 42 were successfully weaned. The rates of growth of the third and succeeding generation rats on the S.A.S.-calcium acid phosphate diet have been fully equal to the growth of the first generation animals. A female rat representing the fourth generation, born March 5, 1929, weighed 195 grams on June 10, 1929. On May 30, 1929, at an age of 86 days, she gave birth to a litter of 10, weighing 45 grams. Three of these were raised and had

an average weight of 28 grams at an age of 26 days. Another female representing the 5th generation on the S.A.S.-calcium phosphate ration, born on August 28, 1929, weighed on November 5, 1929, 175 grams. On November 2, at the age of 66 days she gave birth to a

TABLE 2.

Reproduction on S.A.S.-Calcium acid phosphate diet.

Pair no.	Date: Birth of young	No. young	Av. birth, wt. grams	Weaning age, days	Av. weaning, wt. grams	Total litter at weaning, grams	No. weaned
1	March 2, 1929	11	5	24	29	262	9
	April 6, 1929	12	5.8	20	38	188	5
	May 20, 1929	13	4.5	20	28.5	171	6
	July 5, 1929	9	5.4	21	30.0	242	8
	August 2, 1929	11	5.2	20	25.6	282	11
	September 4, 1929	7	5.3	21	39.3	275	7
	October 1, 1929	9	5.6	21	39.5	237	6
	October 25, 1929	7	5.5	All died			
2	March 5, 1929	12	5.6	21	30.0	150	5
	April 27, 1929	11	5.8	20	35.0	278	8
	June 10, 1929	13	5.3	21	26.0	340	13
	July 22, 1929	4	6.4	20	33	231	7
	August 16, 1929	7	5.4	21	33	198	6
	September 17, 1929	6	6.0	21	41	246	6

litter of 10 weighing 58 grams. Nine of these were raised. At 20 days of age their average weight was 26 grams. Control rats were not carried beyond the first generation because of failure to breed. Other rats in our laboratory, not used in this experiment, but fed a ration similar to that here employed but without baking powder residues have not excelled the reproductive records just cited. We conclude from our experience that the ingestion of S.A.S. calcium acid phosphate residue in the amounts used in these experiments does not impair the reproductive function. The use of tartrate powders likewise probably had no effect on reproduction in our experiments. Unfortunately we lost by infectious disease all our second generation tartrate animals at a time when the first generation were too old to reproduce.

Our intention was to determine the maximum length of life of the rats on the several diets. The prevalence of lung infections was so great, however, that longevity on all the diets could not be determined with any satisfaction. During the latter part of December 1928, an epidemic of undetermined origin carried off 33 animals which were

nearly all of those remaining at that time. The only ones that survived were three on the high S.A.S. diet, one on the intermediate tartrate diet, and two second generation S.A.S. animals. The four first generation animals that survived this epidemic were kept until they reached an age of 21 months when they were killed for post mortem examination. The average length of life on the various rations was: control 10 months for the males and 7 months for the females; for the S.A.S. acid phosphate diets 10 months for the males and 8.7 for the females; and for the tartrate diets 6.7 months for the males and 10 months for the females. There is no evidence, therefore, that the ingestion of the baking powders shortened the life span of rats fed thereon. The average length of life on the highest S.A.S. diet was 9 months for the males and 11 months for the females and for the highest tartrate diet 9 months for the males and 10 months for the females.

Effect of the ingestion of baking powder residues on the non-protein nitrogen of rats' blood. If the ingestion of baking powders is injurious to the tissues of the body the kidney function might be deranged with a consequent rise in the non-protein nitrogen of the blood. To determine non-protein nitrogen the blood samples were collected by stunning the rats by a sharp blow on the head and immediately severing the blood vessels of the neck. Non-protein nitrogen was determined by the Folin-Wu method.

Non protein nitrogen in rats' blood. Milligrams per 100 cc.

Diet	Sept. 6, 1927	Nov. 4, 1927	Jan. 4, 1928	Mar. 4, 1928	June 4, 1928	Average
Control	44	43	37.2		36	40
Control + 1 g. S.A.S. to 223 g. food	41	40	40	48	38	42
Control + 1 g. S.A.S. to 56 g. food	23	33	40	43	32	34
Control + 1 g. S.A.S. to 669 g. food	30	41	29	48	36	37
Control + 1 g. tartrate to 116 g. food	44	49	29	39	43	41
Control + 1 g. tartrate to 29 g. food	19	35	30	50	38	34
Control + 1 g. tartrate to 340 g. food	38	41	38	40	41	40

The results show that the non-protein nitrogen of the blood of rats that are ingesting small to large amounts of baking powder residues does not differ appreciably from that of control rats. The non-

protein nitrogen of the blood, therefore, does not give any indication that kidney function is impaired in animals that are ingesting small or relatively large amounts of baking powder residue.

In conjunction with the determination of the rate of growth, longevity, and reproduction, it was decided that valuable information might be obtained by observing any pathological changes occurring in the kidneys of the animals used in the experiment. It was therefore decided that one animal from each of the feeding groups should be killed at stated intervals, during the course of the investigation, and that macroscopic as well as a microscopic examination be made of the kidneys of each rat killed. This had been done, the first series of rats being killed 45 days following the beginning of the special feedings, while those killed at the close of the experiment had been on their diets for a period of twenty-one months.

Each rat was killed by a blow on the head; the jugular vein was then severed and blood collected for the chemical test. Immediately after this, a postmortem was done and all viscera carefully examined macroscopically. Portions of each organ were placed at once in a fixing solution of 5 per cent formalin in Zenker's solution. In this way the tissues were in the fixing solution in a perfectly fresh condition, in many instances before the heart beats had entirely ceased. After fixation, parts of the fixed tissues were selected and embedded in paraffin in the usual way, after which microscopic sections were cut and stained by the hematoxylin-eosin method. Slides were prepared in this manner from the following organs of each rat; kidney, adrenal gland, liver, spleen, stomach, intestine (duodenum), testis, lung, heart and in the later part of the series the ovary.

The animals and the tissues were submitted to the pathologist by numb[illegible]only, so that during the entire examination he had no knowledge [illegible]e kind or amount of food the animals under observation had receiv[illegible] During the course of the experiment a number of animals were k[illegible]y a pulmonary infection characterized by multiple abscesses of the lung, to which Donaldson (7) states the albino rat is subject. These deaths were about equally distributed among the various groups of animals, the food bearing no relationship to the deaths. An attempt was made to identify the causative organism but no satisfactory results were obtained. None of the rats found dead in their cages and none that were found to be infected at the time of autopsy were included in the series here reported.

Upon examining the kidneys their gross appearance as to size, color, texture, relationship of capsule and the amount of cortical

substance were considered. No changes were found in the kidneys of the animals examined whereby the organs in one group could be distinguished from those of the remaining groups. In the microscopical examination pathological changes were looked for in the tubular epithelium, both nuclear and cytoplasmic, the glomeruli, the glomerular capsules, the blood vessels and in the stroma or interstitial framework of the organ. Here again the kidneys from the animals of the various groups were closely similar, and, while there were slight structural differences to be seen in individual sections examined, when grouped according to the various methods of feeding, there was no single group that could be selected because of any marked or constant variations in either tubular epithelium, glomeruli or stroma. The histological variations were so slight that the kidneys of those animals receiving the highest amounts of the baking powder residue could, in no instance, be distinguished from those of the control group or from those of other normal animals.

This agrees entirely with the results of Rose and Catherwood (8). In no case did we note the pathological changes described by Seibert and Wells (9) as occurring in the kidney of the rabbit following the administration of the salts of aluminum. It was believed that the rats receiving the larger doses of tartrate baking powder residue might exhibit changes in the tubular epithelium of the kidney and when these expected pathological lesions failed to appear it was thought that perhaps the albino rat was resistant to the action of tartrates. This thought was strengthened when, after a rather careful examination of the literature, no instances were found in which the white rat had been used as the experimental animal in the study of tartrate nephritis. Therefore, it was decided to test this point and a series of rats were subjected to varying doses (0.1–0.4 gm. per 100 gms. body weight) of sodium potassium tartrate administered subcutaneously by hypodermic injection. This proved that the failure to find pathological changes in the kidneys was not due to the insusceptibility of our experimental animals, for within 48 hours the kidneys of the animals receiving the larger doses showed a very pronounced degeneration of the tubular epithelium throughout the cortical portion, resembling in all regards the nephrosis described as occurring in other animals following the administration of tartrates (Underhill, Wells, and Goldschmidt, 10), (Karshner and Dennis, 11).

At the close of the experiment there were four of the original rats remaining. Three of these were from the group receiving the highest ration of the S.A.S. baking powder residue and the fourth was from the

intermediate group of tartrate fed animals. A fifth animal examined at the expiration of the experiment was one of the second generation receiving the highest S.A.S. ration. The microscopic examination of the kidney sections prepared from these animals, some of which had been on a continuous diet containing approximately 2 per cent of S.A.S. calcium acid phosphate baking powder residue for a period of 21 months revealed no pathological change by which they could be distinguished from the sections taken from the animals in the earlier period of the experiment or from the control animals.

Summary.

1. The ingestion of sodium aluminum sulfate-calcium acid phosphate baking powder residue by white rats in varying amounts up to a dosage represented by approximately 2 per cent of the diet had no appreciable effect on the rate of growth, maximum adult size, longevity, reproduction and non-protein nitrogen of the blood. We conclude, therefore, that it was without injurious effect.

2. The ingestion of tartrate baking powder residue by white rats in varying amounts up to about 4 per cent of the diet had no marked effect on rate of growth, maximum size, longevity, reproduction and non-protein nitrogen of the blood. We conclude, therefore, that it was without marked injurious effect.

3. Six generations have been raised on rations containing approximately 2 per cent S.A.S. baking powder residue for the first and second generations and 4 per cent for the succeeding generations. The rate of reproduction, the number of young successfully weaned and the rate of growth of the young show that the S.A.S.-containing ration is fully equal, as concerns reproduction in the white rat, to similar rations containing none of this baking powder.

4. Post mortem examinations were held upon all of the animals of the experiment. There were 46 animals killed for the purpose of autopsy. The shortest period of feeding for animals so killed was 46 days, the longest period 21 months.

5. The microscopic examination of the kidneys of the animals, fed after the manner described in this paper, failed to reveal any pathological changes by which the kidneys of the various groups could be differentiated. Neither the length of time during which the feed was consumed nor the proportions of the ingredients of the food itself brought about sufficient change to warrant a pathological diagnosis of any sort.

6. The kidneys of albino rats, fed for a period of 21 months upon a

feed containing approximately 2 per cent of S.A.S. baking powder, presented no gross or microscopic lesions whereby they could be distinguished from the kidneys of rats receiving the basic ration containing no baking powder residue.

Bibliography.

1. Smith, E. E. Aluminum Compounds in Food, Paul B. Hoeber Inc., New York, 1928.
2. McCollum, Rask and Becker. Jour. Biol. Chem., *77*, 753–68, 1928.
3. Underhill, F. P., and Peterman, F. I. Amer. Jour. Physiol., *90*, [illegible]
4. Myers and Mull. Jour. Biol. Chem., *78*, 605–13, 1928.
5. United States Department of Agriculture. Bull. 103, 1914.
6. Schaeffer, Fontes, Breton, Oberling and Thivolle. Bull. soc. hyg. aliment, *16*, 1–24, 1928.
7. Donaldson, Henry H. The Rat, p. 327, Philadelphia, 1924.
8. Rose, Wm. C., Catherwood, F. L. Jour. Nutrition, 1929, II, 2. 161.
9. Seibert, F. B., and Wells, H. G. Arch. Path., *8*, 2, 230, 1929.
10. Underhill, F. P., Wells, H. G., and Goldschmidt, S. Jour. Exp. Med., 1913, *18*, 322.
11. Karshner, H. T., and Dennis, W. Jour. Exp. Med., 1914, *19*, 259.

J. Biochem J 26. 833-845 (1932)

CII. THE BIOCHEMISTRY OF ALUMINIUM.

III. EFFECT OF ALUMINIUM ON GROWTH AND REPRODUCTION IN THE RAT.

IV. THE OCCURRENCE OF ALUMINIUM IN THE THYROID.

V. INTESTINAL ABSORPTION OF ALUMINIUM IN THE RABBIT.

By KENNETH MACKENZIE.

From the Rowett Research Institute, Aberdeen.

(*Received March 31st, 1932.*)

III. EFFECT OF ALUMINIUM ON GROWTH AND REPRODUCTION IN THE RAT.

The use of rats for testing the biological importance or significance of foodstuffs and accessory factors is of comparatively recent introduction. This paper deals with the effect of aluminium in the rat when various essential constituents of the diet are varied, as well as with its effect when added to normal diets.

Other workers who have tested the effect of aluminium on rats include Myers and Mull [1928], McCollum, Rask and Becker [1928], Rose and Catherwood [1929-30] and Massatch [1930]. In all these experiments, aluminium added to the ration in amounts up to 0·1 % produced no effect when the diet was otherwise adequate. Lyman and Scott [1930] reported that no change in blood constituents (non-protein nitrogen, *etc.*) occurred on such diets. Kraft [1930] stated that the digestibility of food was not impaired by addition of aluminium. *Post mortem* examinations performed by most of these workers indicated no pathological effects, and very slight or negative absorption of aluminium.

Slight positive evidence was adduced by Daniels and Hutton [1925], who reported that rats fed on milk did not reproduce for more than two generations, but that additions of soya bean ash or the equivalent inorganic salts (aluminium, manganese, silicon and fluorine) caused satisfactory reproduction for six generations. The effect of the separate elements was not tested; but Orent and McCollum [1931] have stated that manganese is essential in reproduction.

Toxic effects due to aluminium have been reported by Schaffer *et al.* [1929] working with mice, particularly as to impairment of reproduction, histological examination of the ovary showing that this was markedly affected.

The adequacy of the diet and excessive extent of the aluminium dosage has been questioned by McCollum *et al.* [1929; see also reply by Schaffer *et al.*, 1929]. Bertrand and Serbescu [1931] have reported that the toxicity of aluminium salts injected parenterally is less than that of nickel or copper in the case of rats and rabbits.

Experimental.

It was originally intended to test the effect of raising several generations of rats on a diet entirely devoid of aluminium, as well as to test the effect of adding various levels of aluminium salts to the diet. Unless the former procedure is carried out, effects due to traces of aluminium cannot be excluded. A synthetic ration was employed, each component of which could be purified and tested separately, but certain of these—the vitamin-containing materials and particularly yeast—offered great difficulty. It is almost impossible to eliminate the inorganic constituents from yeast or marmite, yet to prepare vitamin B concentrates, especially at the time when these experiments began, was considered to be beyond the scope of this work. Hence the problem became one of limits—a variety of suitable constituents being tested for aluminium, and those containing the minimum quantity selected for use. Samples of these materials were ashed and examined spectrographically, and Table I gives the maximum and minimum amounts found in each constituent used over a period of 18 months.

Table I. *Maximum and minimum amounts of aluminium found in foodstuffs.*

Material		Starch	Caseinogen	Yeast	Butter	Food mixture
Aluminium in parts per million	Maximum	0·3	0·2	3·7	1·5	0·5
	Minimum	0·1	—	1·2	—	—
Lowest limit observable*		0·1	0·05	0·6	0·1	0·4

* About 1 part of aluminium in 20,000 parts of inorganic ash can be detected, so that the ash content of each substance is the limiting factor in the spectrographic determination of aluminium. The method can be made roughly quantitative [see McCollum *et al.*, 1928].

As the food mixture contained only small portions of yeast (see Table II of rations for details) the limits of aluminium content could be much higher than with the other constituents without unduly raising the total aluminium. Samples of the control ration, therefore, never contained more than 1 part of aluminium in 2 millions. Effects of amounts smaller than this, if such exist, are excluded from consideration here.

The upper limit of aluminium for experiments where this formed a part of the diet was fixed at about 0·08 %. This amount may be administered in the food in the form of phosphate (baking powder residues) or sulphate (alums) without producing cathartic effects, and is analogous to the amount ingested by the human being when living upon a mixed diet [Smith, 1928].

Exp. 1. Thirty rats weighing 50–100 g. were used, 20 receiving the synthetic diet only, the other 10 with the addition of 0·1 % aluminium as soda alum. (The rations are set out in Table II.) They were allowed a[illegible]h food as they could consume, together with water. The animals were a[illegible]placed in separate glass cages (large battery jars), with wire mesh mats covering the bottom, under which was placed a pad of several layers of filter-paper, the mouth being fitted with a glass plate with gaps for ventilation. Subsequently, the rats were placed in groups of three in the same cages, as they were unfavourably affected by isolation. In later experiments, the use of glass cages was given up, since, although they afforded an environment free from aluminium, this precaution was unnecessary owing to the presence of aluminium in the food, and in general working, glass cages were found to be fragile in use, and extravagant in space and labour. They were only used for special purposes subsequently (*e.g.* metabolic experiments, as described elsewhere [Mackenzie, 1931]).

Table II. *Diets.*

(All components expressed as percentages.)

Diet	Yeast	Caseinogen	Cellulose	Butter	Lard radiostol	Starch	Salt mixture 185	Aluminium* Compound baking powder	Aluminium* Meta
(*a*) Complete:									
A	5	18	2	28	—	43	4	—	—
B	5	18	2	—	—	41	—	2·0†	0·094
C	5	18	2	—	—	39·6	—	3·4	0·1065
(*b*) Vitamin A deficient (?) or absent:									
D	6	22·5	2·5	10	—	54	5	—	—
E	6	22·5	2·5	10	—	51	5	3·0	0·094
F	6	22·5	2·5	—	10	54	5	—	—
G	6	22·5	2·5	—	10	51	5	3·0	0·089
H	6	22·5	2·5	10	—	47·2	5	6·8	0·173
(*c*) Vitamin B deficient (?) or absent:									
V	2	22·5	2·5	20	—	55	—	—	—
W	2	22·5	2·5	20	—	52	—	3·0	0·082
X	0	22·5	2·5	20	—	55	—	—	—
Y	0	22·5	2·5	20	—	52	—	3·0	0·082
Z 1	0	22·5	2·5	—	20	55	—	—	—
Z 2	0	22·5	2·5	—	20	52	—	3·0	0·087

* Actual aluminium content of the food as determined by analysis of samples: not necessarily corresponding to the amount of baking powder residue, which had varying composition and moisture content.

† As soda alum.

The growth curves for rats of Exp. 1 are shown graphically in Fig. 1. The method adopted is to show the average growth for the groups of one sex together with the individual highest and lowest growth curve. It is considered that this gives the fairest computation of the results obtained. Only the growth curves of females are shown in this case, the males showing a general correspondence but about 20 % greater individual weight.

When maximum weight had been reached, and breeding tests were satisfactory, as shown by the production of viable young by each female (see

Table ...nder appropriate experiment) a proportion of the animals were killed ... organs analysed for aluminium, using the colorimetric methods of Myers and Morrison [1928]. Owing to the small amount of material obtained from individual rats, it was necessary to carry out these analyses on groups of five or more animals. The results are shown in Table IV.

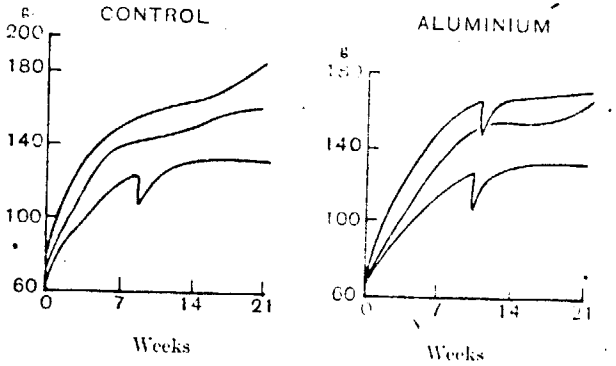


Fig. 1, Exp. 1. Upper curve—heaviest female; lower curve—lightest female; middle curve—average for all females in group.

Table III. *Fertility of animals used in experiments.*

Note. The animals were placed in groups of three, one male and two females as far as possible. If no progeny resulted, the male was considered sterile and a male from another group employed. If only one female produced young, the other was tested with another male before being pronounced sterile. The production of viable young was thus the criterion. Excess males were tested when circumstances allowed.

		Control group				Aluminium group			
Exp.	Sex	Total number	Fertile	Sterile	Not tested*	Total number	Fertile	Sterile	Not tested*
1	♂	4	4	0	—	3	3	0	—
	♀♀	16	12	0	4	7	5	0	2
2	♂	7	6	1	—	8	5	2	1
	♀♀	8	6	0	2	7	7	0	—
1 A (first generation)	♂	7	5	0	2	8	5	0	3
	♀♀	13	10	1	2	10	9	1	—
1 A (second generation	♂	6	4	0	2	8	5	0	3
	♀♀	9	9	0	—	17	10	0	7
1 A (third generation)	♂	7	6	0	1	11	6	0	5
	♀♀	12	8	0	4	9	9	0	—

* Including premature deaths.

Exp. 2. This was a repetition of Exp. 1, but the rats were placed in groups of three in glass cages, and the aluminium was supplied in the form of a decomposed baking powder, containing about 3 % of aluminium. To decompose it, this was heated on a water-bath with half its weight of water, and dried at 100°. The product was added to the ration at a level of 3-3·5 %, replacing an equal weight of starch, but as 50 % of the baking powder itself consists of starch, the variation in this item in the diet was insignificant (see ration C in Table II). The experiment lasted 10 months, and the growth curves, breeding results and analyses appear in the respective ... and Figures.

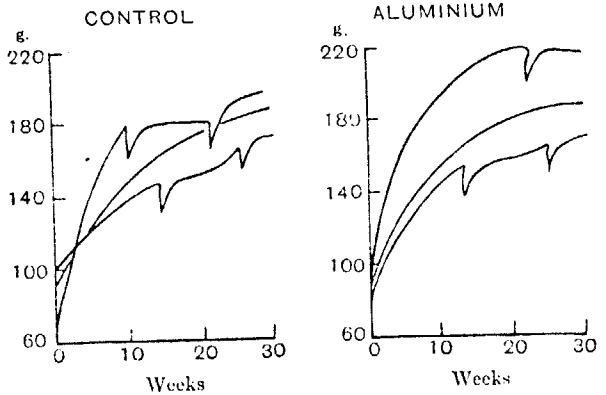


Fig. 2, Exp. 2. Upper curve—heaviest female; lower curve—lightest female; middle curve—average for all females in group.

Exp. 1 A et seq. *Effect of aluminium upon subsequent generations.* Progeny of rats employed in Exp. 1, both control and aluminium-fed animals, were

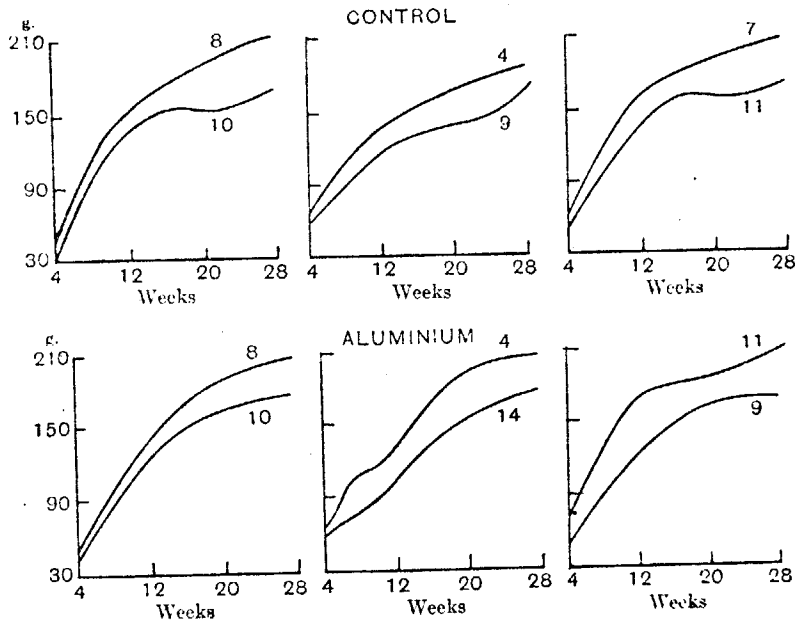


Fig. 3, Exp. 1A. Upper curve—average for all males in group, and number of males. Lower curve—average for all females in group, and number.

used to determine if aluminium at a high or low level could exercise any depressant effect upon growth or fecundity in subsequent generations. The rations used were diets A and C, and three generations were successfully raised. In each generation, about twenty of the young were selected from all

the I[illegible]available, and the remainder discarded. In order to shorten the time [illegible]d by these experiments, breeding was allowed to commence at the earliest possible age, the sexes not being separated, and the first litters were used as representatives of the next generation. This may explain why the growth curves in all generations are on the low side in both groups. Considerable mortality occurred in the second generation owing to extensive and unavoidable changes of temperature in the cage room during the progress of this experiment. In the case of the aluminised groups, five died, and others from the same generation and on the same diet, which had been set aside for metabolic experiments, were brought in to replace them. In these experiments the rats were placed in the usual type of metal cages in groups of five, the diet being supplied *ad lib.* and unlimited breeding being allowed. The results are shown in growth curves, breeding Table and analysis.

As no perceptible effect due to aluminium could be observed when the diet was sufficient for normal requirements, it was decided to make tests under less favourable conditions, *viz.* when the vitamin factors in the diet were reduced to the lowest level sufficient for maintenance, or below. It seemed possible that any differential effect due to aluminium would be more marked under these conditions.

Exp. 3A. Twenty rats were divided into two groups receiving rations D (control) and E (aluminised) respectively. The rats weighed 40–60 g. at the commencement of the experiment. The fat content and vitamin A, supplied

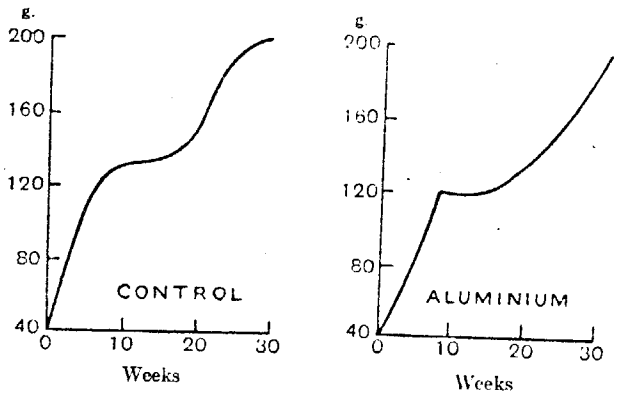


Fig. 4, Exp. 3 A. Low vitamin A (males).

in the form of butter, were thus reduced to one quarter of the normal. Growth did not vary significantly between the groups and was little below normal generally, though the mortality was higher. After 11 months, 7 rats remained in the control groups and 5 in the aluminium group, they were killed and used for analysis. Growth curves and analytical results are shown in Fig. 4 and Table IV.

Exp. 3B. *Effect of avitaminosis-A.* Twenty rats, 10 receiving diet F (control) and 10 receiving diet G (aluminised), initial weight about 100 g., were employed. The ration contained lard instead of butter, t[illegible]luding vitamin A. The growth curves fell off sharply after 6 or 8 w[illegible]and no animal survived 4 months after the experiment began, yet no convincing evidence was obtained that aluminium had any accelerating or retarding effect upon the morbidity. In each group individual growth curves of five individuals, including the extremes (earliest and latest deaths) are shown (Fig. 5).

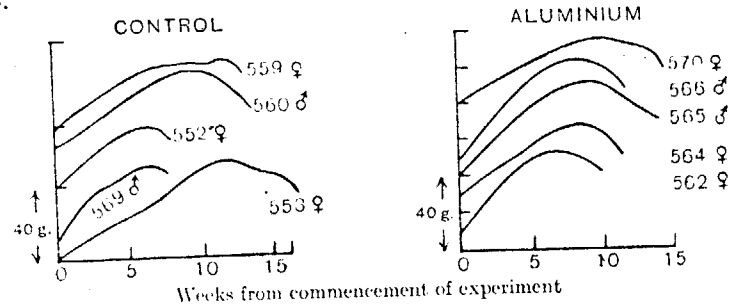


Fig. 5, Exp. 3B. No vitamin A (animals weighed 100 g. to commence).

Exp. 3C. *Effect of feeding high levels of aluminium.* Ten rats, being surplus offspring of rats of Exp. 1, were placed on diet G (containing 1·7 % of aluminium as baking powder residue, *i.e.* aluminium phosphate), and their growth was compared with that of 5 rats placed on diet C, similar in all respects except for the omission of the baking powder. Growth was distinctly below normal, but it was noticeable that the rats did not eat the diet readily, and the consumption per head was much less than in the control group (about 9 g. against 13 g.). The ration had a pronounced saline flavour and apparently induced thirst. Analysis of the animals after 6 months on the diet showed no noticeable storage of aluminium in the organs.

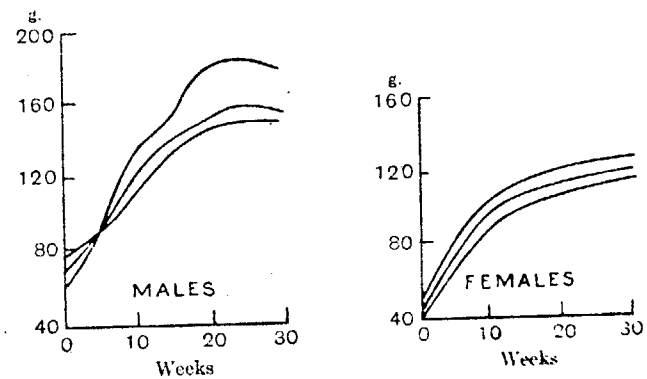


Fig. 6, Exp. 3C. High aluminium diet (highest, average and lowest growths in group).

Exp. 3D. *Effect of limiting the vitamin B complex.* A group of 40 rats, about 40–60 g. in weight, was divided into 4 groups of 10, receiving rations

...(which contained little or no yeast, with and without additions of al... m). They were caged in groups of five, and coprophagy was prevente...sual. A number of the animals exhibited spasmodic inco-ordination in the early stages, and this was treated by the administration of 10–20 cc. of milk to the affected animal. This produced rapid recovery without supplying excessive amounts of vitamin B. Very few of the animals died during the first 2 months, and the growth curves showed steady if small increases—indicating that butter is a sufficiently rich source of vitamin B when fed at a level of 18% to maintain growth in the early stages of life. By the sixth month extensive mortality commenced in the control group, while the aluminium group was very little affected. Under these conditions the baking powder addition may be suspected of some specific sparing action or contributory vitamin. The point was not entirely solved by the next experiment.

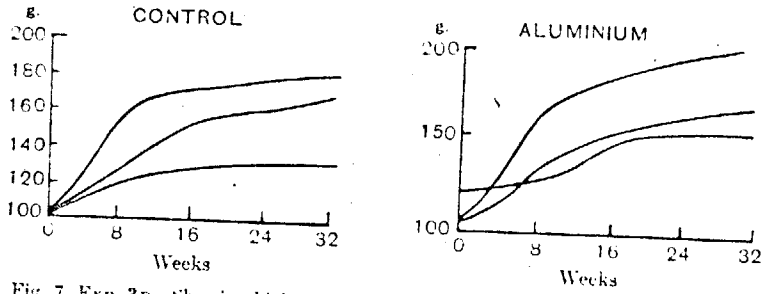


Fig. 7, Exp. 3D. Showing highest, mean and lowest growth curves of the groups.

Exp. 3E. *Effect of aluminium on avitaminosis-B.* Twenty rats were divided into 2 groups of 10, receiving diets similar to X and Y, where lard replaced the butter. Radiostol was also added. The animals were given milk in the early stages whenever inco-ordination was observed, thus effecting a temporary cure, and the experiment was on parallel lines with Exp. 3D. The mortality, as would be expected on a diet entirely deficient in vitamins A and B,

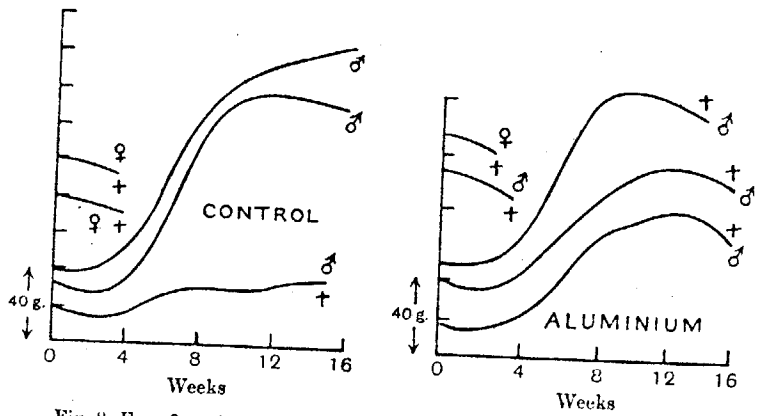


Fig. 8, Exp. 3E. Average weight of animals—50 g. at start; †=death.

was high. In 2 months 7 animals died. The milk supplement was then discontinued, but 8 animals survived after 3 months, and were ...tly resistant to avitaminosis-B. After 4 months typical symptoms of avi...minosis-A set in, and only 2 animals (both controls) survived to the fifth month. These rats had shown unusual growth and vigour throughout, and apart from the exceptions, the 2 groups showed no significant difference [see growth curves of Fig. 8, Exp. 3E].

Discussion.

It is probable that the environment was responsible for the fact that the growth curves in Exps. 1 and 2 were not up to normal, but they showed little difference as between groups on control and aluminised diets. The fertility of rats in these experiments and in the subsequent generations raised from rats of Exp. 1 also show no differential effect. Exp. 3B, in which high levels of aluminium were fed and some stunting of growth resulted, has some resemblance to the effects obtained by Schaffer *et al.* [1928]. In this case it was notable that the difference in the amount of food consumed would account for the poor growth, the rats taking very little more of it than would suffice for maintenance. To obtain clear-cut results it would be desirable (*a*) to feed a control group with the control diet at the same level as that at which the experimental rats would consume their ration, and compare the growth curves; (*b*) to feed a form of aluminium which is tasteless and non-cathartic, pure aluminium phosphate would probably be suitable, so that the diet would be more freely consumed.

Under present circumstances, the question as to whether the stunting effect is due to a specific action of aluminium, or a general lack of balance in the diet, must be considered an open one. Exp. 3D, in which an apparent lengthening of life resulted from the aluminium addition, may be considered to be due to a specific action of aluminium, but the other components of the baking powder—starch and albumin—must not be overlooked as a possible source of vitamin B. On the whole, the avitaminosis experiments give no reason to suppose that aluminium has any action one way or the other.

The analytical results suggest that some absorption of aluminium by the liver occurs on aluminised diets. The amounts do not seem to increase cumulatively with the period of feeding, and are quite comparable with the amount found in control animals [see also Tourtellotte and Rask, 1931].

Conclusions.

(1) Aluminium additions to the diet have no perceptible influence
  (i) when the diet is adequate for growth and reproduction in the rat;
  (ii) when the diet is insufficient for growth or maintenance;
  (iii) when fed to several successive generations of rats.

(2) Aluminium absorption from such diets appears to be exceedingly small.

IV. THE OCCURRENCE OF ALUMINIUM IN THE THYROID.

It has been reported by Underhill *et al.* [1929] that aluminium tends to accumulate in the thyroids of dogs when the animals are on an aluminium diet, amounts up to 0·07 mg. having been found in a single thyroid. Recently Tourtellotte and Rask [1931] have questioned these results. Since 1929, as occasions have offered, samples of thyroid have been secured from normal, aluminium-fed and iodine-fed animals in this Institute, in an endeavour to determine whether aluminium is present in the thyroid, and if it can be connected in any way with iodine metabolism. Iodine and aluminium have been determined in each sample (except in two cases where the total amount of tissue would not suffice for aluminium assay, in which case iodine alone was determined). The aluminium content was determined by the colorimetric method using aurintricarboxylic acid [Myers and Morrison, 1928]; the iodine by the method of Leitch and Henderson [1926]. I desire to thank Miss Stuart of this Institute for the iodine determinations referred to below.

(*a*) *Iodine and aluminium in the thyroids of pigs employed for aluminium experiments* [see Mackenzie, 1931].

Pig No.	Diet	Weight of thyroid g.	Iodine in thyroid g./100 g.	Aluminium in thyroid mg./100 g.
2474	Al less than 2 parts per million	4·81	0·155	0·04
2477		4·11	0·165	0·05
2480		2·6 (part)	0·189	0·9
2475	Above diet *plus* 0·1 % aluminium	5·48	0·193	2·63*
2479		4·51	0·202	—†
2488		8·39	0·241	0·02

* Final product showed iodine coloration—result doubtful.
† Results below 0·02 are not reliable, and are left blank.

(*b*) *Iodine and aluminium in the thyroids of pigs employed for iodine experiments.*

Groups of 5 pigs received (I) control ration adequate for normal growth, (II) same diet + potassium iodide, (III) same diet with ultra-violet irradiation, (IV) same diet, with potassium iodide and ultra-violet irradiation.

Group of 5 pigs receiving	Total weight thyroids g.	Iodine in thyroids g./100 g.	Aluminium in thyroid mg./100 g.
Normal diet	18·2	0·118	0·02
,, +KI	29·4	0·238	—
,, +U.V.I.	22·7	0·202	0·06
,, +KI+U.V.I.	34·1	0·097	0·03

Note. The thyroid material had been dissolved in dilute alcoholic potassium hydroxide for iodine determination, and stored in glass bottles. It was anticipated that aluminium might be present owing to the action of the potash on glass, which usually contains aluminium. But the results show that the aluminium content, from whatever source derived, is extremely small.

(*c*) *Other samples of thyroid from pigs.*

Pig	Weight of thyroid g.	Aluminium mg./100 g.
Normal, 6 months old	3·8	0·12
Depancreatised 2 months previously ...	2·1	0·07
Depancreatised 1 day previously ...	4·7	0·05

(*d*) *Iodine in thyroids of rats receiving aluminised diets.*

The thyroid in the rat is too small to be analysed for aluminium by any of the methods now in use. It was considered advisable to find the iodine content of some thyroids in the case of rats used for aluminium experiments, as described above, to see if any correlation might exist. The thyroids of rats in Exps. 1 and 2 were analysed by Miss Stuart, and the results follow:

Exp. No.	No. of rats examined	Average weight of thyroid g.	Iodine content of thyroid g./100 g.
1 (control aluminised)	10	0·017	0·042
	5	0·015	0·061
2 (control aluminised)	6	0·016	0·027
	7	0·019	0·026

These results indicate that

(i) aluminium occurs at only a very low level in the thyroid;
(ii) there is no correlation between aluminium and iodine in normal metabolism.

V. INTESTINAL ABSORPTION OF ALUMINIUM IN THE RABBIT.

The presence of small quantities of aluminium in the normal diet of men and animals [Smith, 1928], and the presence of aluminium in very minute quantities in the tissues [Myers and Mull, 1928], are both well established facts which have not been satisfactorily correlated. It is established that certain aluminium compounds, when present in the food, are converted into a soluble form by the action of the gastric juice [Myers and Killian, 1928] and it seems reasonable to suppose that in the upper part, at least, of the small intestine, absorption of the aluminium ions may be expected to occur as is the case with calcium, sodium and potassium, though in the lower parts of the intestine aluminium appears to be precipitated [Massatch, 1930]. The absorbed aluminium might be conveyed by the portal blood to the various organs, notably to the liver, and it has been observed that the aluminium content of the liver is greater in aluminium-fed animals than in controls. Though the amounts are in all cases very small [Underhill *et al.*, 1929] the increase is definite, and can hardly be explained on any other ground. But the presence of detectable amounts of aluminium in the blood after ingestion of aluminium compounds has rarely been observed, the findings of all workers being negative except those of Underhill *et al.* [1929]. These workers, using an exceptionally sensitive method for detection of alum, found that after some +

absent from the blood of normal animals, and even when present the initial amount was not always increased by aluminium ingestion, while in some cases a fall was observed.

Myers and Morrison [1928] injected a known amount of an aluminium compound, usually the sulphate or chloride, into a washed intestinal loop in dogs, and found it possible to recover the aluminium, within the limits of experimental error, after a period of 1 to 4 hours. They pointed out, however, that the aluminium was recovered as a precipitate on the mucosa, and it seems certain that the normal reaction of the intestine would convert most aluminium salts to the hydroxide, which is colloidal and probably non-absorbable.

In the present experiments, a known amount of aluminium in the form of a solution of aluminium tartrate was injected into the ligatured intestine of a rabbit (which had received an aluminium-free diet for one week previous, and had fasted for 24 hours before the experiment) by the method due to Magee and Macleod [1928]. The rabbit, anaesthetised with urethane and ether, was opened up to expose the viscera, and the intestine tied off at the oesophagus, the ileal extremity being cut and sutured to a discharge tube. A small cannula was inserted below the ligature, and 10 cc. of aluminium tartrate solution (1 mg. Al per cc.) at approximately p_H 8 were injected. After an interval varying from 15 to 30 minutes, about 20 cc. of blood were drawn from the portal vein by hypodermic syringe; a second sample was drawn after a further period of 10 to 15 minutes. The animal was then bled to death through a cannula in the carotid artery, and subsequently the liver and entire small intestine were dissected out. These samples of blood and tissue were ashed in silica basins and the aluminium content was determined by the colorimetric method. In addition to 7 animals thus used, 2 others were used as controls, 10 cc. of plain saline being injected into the intestine, the other operations being as before. The results are shown in Table IV.

Table IV. *Aluminium found in the blood of rabbits after injection of aluminium tartrate into intestine.*

Exp. No. ...	1	2	3	4	5	6	7	Control 8	
Aluminium injected into intestine (mg.)	11·05	10·8	10·4	11·05	11·05	10·3	11·05	0·0	0·0
Aluminium found (mg./100 g.): (*a*) in portal blood									
1st sample	0·017 (20)	0·047 (30)	0·000 (15)	0·016 (15)	0·000 (15)	0·000 (25)	0·000 (25)	0·000	0·0
2nd sample	0·049 (35)	0·000 (45)	0·021 (45)	*	0·000 (25)	0·000 (35)	0·000 (40)	—	—
(*b*) in systemic Blood	0·000 (40)	0·000 (50)	—†	—	5·60‡	0·000 (40)	0·083 (45)	0·000	0·0
(*c*) in the liver	0·56	0·86	—	0·42	0·79	0·55	1·15	1·12	0·7
Aluminium found in intestine (mg.)	1·48	8·82	9·04	11·3	9·6	7·73	9·6	0·67	0·5
Percentage recovered from intestine	13·5	81·7	86·9	102·2	87·7	75·1	87·7	—	—

Average percentage recovered in six experiments ... ... 86·8

The figures in brackets following the blood figures are the times (mins.) from the initial injection at which the blood sample taken.

* This animal died 25 minutes from commencement.
† This animal died 45 minutes from commencement.
‡ This animal died 30 minutes from commencement. The systemic blood sample was obtained from the body cavity, and possibly contaminated by the liquid injected into the intestine—the actual amount of aluminium present in 10·7 g. of blood

Discussion.

The average recovery in 6 experiments varied from 75·1 % to 102·2 % with a mean value of 86·8 %. The first experiment showed an extremely low recovery of aluminium from the intestine, possibly due to leakage at the site of injection. It stands alone and has been ignored in computing the average. In the 2 control animals, the aluminium level in the intestine was about 5 % of that found in the experimental animals, showing that 7 days on an aluminium-free diet was not sufficient to remove all traces of aluminium from the intestine. Hence it appears probable that the average recovery of aluminium injected is only about 82 %.

If the aluminium content of the portal blood is compared with the amount present in the intestine, it does not seem probable that aluminium is readily absorbed by the intestine. The amount circulating in the blood at any time is extremely small, but it is undeniable that aluminium may be, and frequently is, conveyed in the blood-stream.

Summary.

Injection of aluminium salts into the intestine of rabbits does not cause any appreciable absorption of aluminium into the blood-stream. The aluminium injected can be largely recovered by analysis of the intestine.

REFERENCES.

Bertrand and Serbescu (1931). *Compt. Rend. Acad. Sci.* **193**, 128.
Daniels and Hutton (1925). *J. Biol. Chem.* **63**, 143.
Kraft (1930). *Amer. J. Hygiene*, **12**, 283.
Leitch and Henderson (1926). *Biochem. J.* **20**, 1003.
Lyman and Scott (1930). *Amer. J. Hygiene*, **12**, 271.
Mackenzie (1931). *Biochem. J.* **25**, 287.
Magee and Macleod (1928). *J. Physiol.* **67**, *Proc.* xvii.
Massatch (1930). *Biochem. Z.* **220**, 239.
McCollum, Rask and Becker (1928). *J. Biol. Chem.* **77**, 753.
—— —— —— (1929). *Bull. Soc. Hyg. Alim.* **17**, 65.
Myers and Killian (1928). *J. Biol. Chem.* **78**, 595.
—— and Morrison (1928). *J. Biol. Chem.* **78**, 615.
—— and Mull (1928). *J. Biol. Chem.* **78**, 605.
Orent and McCollum (1931). *J. Biol. Chem.* **92**, 651.
Rose and Catherwood (1929–30). *J. Nutrition*, **2**, 155.
Schaffer, Fontes and Oberling (1928). *Bull. Soc. Hyg. Alim.* **16**, 1.
—— —— —— (1929). *Bull. Soc. Hyg. Alim.* **17**, 45.
Schwarze, Murphy and Hann (1930). *J. Nutrition*, **2**, 235.
Smith (1928). Aluminium compounds in food. (Hoeber, N.Y.)
Tourtellotte and Rask (1931). *Amer. J. Hygiene*, **14**, 225.

J Biological Chemistry 77: 753-769 (1928)

A STUDY OF THE POSSIBLE RÔLE OF ALUMINUM COMPOUNDS IN ANIMAL AND PLANT PHYSIOLOGY.

By E. V. McCOLLUM, O. S. RASK, and J. ERNESTINE BECKER.

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

Plate 1.

(Received for publication, February 23, 1928.)

Aluminum is the most widely and abundantly distributed metallic element in the earth's crust, of which it is estimated to represent 7.3 per cent, whereas iron and calcium represent only 4.2 to 3.5 per cent respectively. Accordingly, aluminum must have been intimately associated with living matter throughout the ages. In recent years this element has assumed an increasingly important and conspicuous rôle in the human environment due to its use in the purification of water, in cooking utensils, in baking powders, and in medicinal products. Therefore, serious consideration has been given to its possible biological significance and to the effects of its salts in the diet.

Langworthy and Austen (1), Gonnermann (2), Bertrand (3), and others, have reported the presence of significant amounts of aluminum in a long list of plant and animal products. Osborne and Mendel (4) have reported better growth in white rats from diets containing "artificial protein-free milk" to which had previously been added traces of iodine, manganese, fluorine, and aluminum, than from the same "artificial protein-free milk" not containing these elements. Daniels and Hutton (5) have suggested as a result of experimental studies that aluminum may be essential to reproduction.

Such data as the above suggest that aluminum possesses definite physiological functions and that this element is an indispensable dietary constituent.

A different view is held by Gies (6) and his coworkers. They have since 1905 conducted repeated investigations of the dietary

properties of aluminum compounds. As a result they seem convinced that aluminum compounds when present in the diet are absorbed out of the gastrointestinal tract and carried in the blood stream to different parts of the body with harmful effects.

In contrast to the findings of Gies and his coworkers are those of the Reference Board of Consulting Scientific Experts (7), of which Ira Remsen was chairman. These investigators studied the effects on human subjects of a dietary containing biscuits baked with the sodium aluminum sulfate type of baking powder. From these studies they concluded that aluminum residues present in biscuits baked with the sodium aluminum sulfate type of baking powder have no harmful effects on metabolism. Similar conclusions have more recently been expressed by Schmidt and

TABLE I.

Parts of Aluminum per Million.

Worker.	Date.	Egg white.	Wheat.	Corn.	Potatoes.
Theile, R.	1867	762.0			
Penney, M.D.	1879		6000–25000		
Myers, V. C., and Voegtlin, C.	1914		450	1700–1800	200
Gray, P. R.	1924	0.2–0.4	7.4–5.9	9.7–11.2	3.3
Sullivan, B., and Near, C.	1927		3.0		

Hoagland (8). These investigators found, furthermore, that all aluminum salts ingested were excreted in the feces.

The wide divergence of views and conclusions expressed by the above investigators on the biological and dietary significance of aluminum may be due in part to faulty methods of determining aluminum. It is interesting to note that in a chronological compilation such as is given in Table I there tends to be a progressive decrease in the amount of aluminum reported to be found in the different biological products indicated.

Since analytical chemistry has undergone improvement during the period covered by Table I, it may be assumed that the earlier published data are too high. In 1901 Langworthy and Austen published a compilation of data on the biological distribution of

aluminum. In the introduction of this publication these writers state:

"In the older investigations, particularly those dealing with the mineral constituents of plants, data regarding aluminum are more abundant than in later works, and doubtless some of the aluminum reported came from impure reagents, from dirt contaminating the sample, or some similar cause. . . . The greater part of the material included in the compilation does not seem open to that objection, for, as time has progressed, analytical methods and chemical manipulation have improved, and there is no reason why determinations of aluminum made within recent years should not be fairly good."

However, it still seems to be a question whether there exist any trustworthy data on the biological distribution of aluminum. Further improvement in analytical methods or the selection of other and more applicable methods for determining aluminum in biological materials may demonstrate that even the most recent values in Table I are also too high.

Several difficulties may be anticipated in determining aluminum contents of biological materials by chemical methods. Undoubtedly the most serious of these difficulties is that of preventing contamination by aluminum from outside sources. Due to the wide-spread occurrence of aluminum, its complete absence from the best available chemical reagents and from the laboratory environment can hardly ever be achieved or assured even when the most rigid precautions are observed. Accordingly, it is always exceedingly difficult if not impossible for the analyst who uses chemical methods for determining aluminum in such small amounts as may be expected to occur in biological matter to produce data from which are precluded all possibilities of influence by aluminum from outside sources. In using chemical methods for determining aluminum in biological material there will therefore invariably be the probability that part or all of the aluminum reported was due to unavoidable contaminations. For ascertaining the presence or absence of small amounts and also for approximate estimations of such amounts of aluminum, the spectrographic method has the following advantages over all chemical methods: (1) sensitiveness; smaller amounts of aluminum can be detected by it; (2) specificity; the possibility of confusing other substances for aluminum is entirely eliminated; (3)

simplicity and rapidity of manipulations; (4) absence of all chemical reagents except atmospheric oxygen. Such conditions reduce to the lowest possible point all chances of aluminum contamination.

In view of these considerations the spectrographic method was used in this investigation. The details of the technique were as follows: The material was ashed in silica dishes supported on silica triangles over Bunsen flames. The spectrum of the resulting ash was excited in a 20,000 volt condensed spark between vertical copper electrodes by placing 20 to 30 mg. of the ash in a hollow of the lower electrode. The secondary circuit contained a self-induction coil which served to reduce the intensities of the lines due to air. The spectrum so produced was dispersed and recorded on plates by means of a Hilger E 1 quartz prism spectrograph. In the investigation reported in this paper this spectrographic technique was employed in ascertaining the biological distribution of aluminum and in conjunction with feeding tests for ascertaining the dietary properties of aluminum salts.

The *raies ultime* of aluminum are, according to de Gramont (9), the lines 3944.0 and 3961.5. These numbers are the names of the lines and their wave-lengths in Ångstrom units. The line 3961.5 is the first *raie ultime* because a smaller quantity of aluminum is required to produce it than any other line in the aluminum spectrum. This line persists after all the other aluminum lines have disappeared as a result of a progressive dilution of aluminum in the spectral source. The line 3944.0 is the second *raie ultime* because it is second to 3961.5 in the above respects.

It was obvious from the work of de Gramont that the purposes of the investigation could be served adequately and most conveniently by confining spectral examinations to the above two lines. A few preliminary spectrographic experiments of our own showed this to be so. Accordingly, only a portion of the spectrum sufficient to include these and a few adjoining lines has been reproduced in this paper.

In order to facilitate the identification of lines 3944.0 and 3961.5, and also in order to demonstrate the absence of these lines from the copper electrodes, all spectrograms were produced by means of a Hartman diaphragm. The operation of the Hartman diaphragm is illustrated by Fig. 1, which represents two typical

spectrograms. In each spectrogram the upper spectrum is that of the empty electrodes. The middle spectrum, or spectrum of the unknown, is that of the same electrodes with 20 to 30 mg. of the ash to be investigated in the lower electrode. The third or control spectrum is the same as or a continuation of the middle spectrum except that 1 drop of a 0.1 per cent solution of aluminum in form of the chloride was placed on the lower electrode previous to the third exposure and of course after photographing the middle spectrum. The two short and relatively heavy lines near the center and in the third or bottom spectrum are 3944.0 and 3961.5, the former being on the left and the latter on the right. The absence of these lines in the upper spectrum is evidence or proof that the electrodes do not contain aluminum. Their absence from the middle spectrum is evidence of the absence of aluminum in the ash, which was placed in the lower electrode.

In order to ascertain the sensitiveness of this spectrographic technique, or in order to ascertain the smallest quantities of aluminum which could be detected by us, under conditions of this investigation, the spectrograms in Figs. 2 and 3 were prepared. Each of the three spectrograms in Fig. 2 represents ash of 10 gm. portions of the same fresh whole egg preparation. Another and different whole egg preparation was used as a source of ash for the three spectrograms in Fig. 3.

The upper or first spectrogram in Fig. 2 is that of the ash of whole egg. In the middle spectrum of this spectrogram no trace of either aluminum line is present. Accordingly, so far as can be ascertained by this spectrographic technique, whole egg contains no aluminum. The second spectrogram in Fig. 2 is of the ash of 10 gm. of whole egg to which had been added previous to ashing, 1 ml. of a solution containing 0.001 per cent of metallic aluminum in form of the chloride. The egg previous to ashing, therefore, contained 1 part per million of aluminum. In the middle spectrum of this second spectrogram it can be observed that this concentration of aluminum (1 p.p.m.) was sufficient to produce both aluminum lines, although 3944.0 is rather faint. The third spectrogram in Fig. 3 is that of 10 gm. of whole egg to which had been added 2 ml. of the 0.001 per cent solution of aluminum, making a whole egg preparation containing 2 p.p.m. of metallic aluminum previous to ashing. In this spectrogram the aluminum

lines produced by the 2 p.p.m. are slightly but nevertheless distinctly heavier than those produced by 1 p.p.m. in the second spectrogram. The spectrograms in Fig. 2 show, therefore, that this spectrographic technique is easily capable of detecting 1 p.p.m. of aluminum in biological products. Furthermore, the differences between the second and third spectrograms in Fig. 2 suggest the possibility of approximating the quantity of aluminum present.

Fig. 3 contains spectrograms which show that this spectrographic technique is capable of detecting 0.5 p.p.m. of metallic aluminum. The first spectrogram in Fig. 3 is that of the ash of whole egg without addition of any aluminum. It represents, therefore, the same kind of ash as that represented by the first spectrogram in Fig. 2. In conformity with the first spectrogram of Fig. 2, the first spectrogram of Fig. 3 shows the absence of aluminum in whole egg.

The second spectrogram in Fig. 3 is that of the ash of 10 gm. of the same whole egg preparation used in preparing the first spectrogram. But to these 10 gm. was added previous to ashing 0.5 ml. of a solution containing 0.001 per cent of aluminum. This mixture represents, therefore, 10 gm. of whole egg containing 0.5 p.p.m. of added metallic aluminum. An examination of the second spectrogram in Fig. 3 will show that this concentration, *viz.* 0.5 p.p.m. of metallic aluminum, is sufficient to produce line 3961.6 but not 3944.0. The third spectrogram in Fig. 3 is that of whole egg ash containing 1 p.p.m. of aluminum added in the same manner. As is to be expected, this spectrogram contains both aluminum lines, as does the second spectrogram in Fig. 2.

For purposes of this investigation the following conclusions may be drawn from the experiments represented by the spectrograms in Figs. 2 and 3 concerning the sensitiveness of the spectrographic technique described above for demonstrating the presence or absence of aluminum in biological material.[1]

[1] In examining the spectrograms reproduced in this paper due allowance should be made for the fact that they represent the last reproducing step in a successive series of four. The original spectrum was first reproduced on a photographic negative. The image on the negative was next reproduced on a paper print. The image on the paper print was then reproduced on a half-tone [illegible]. Finally reproduced the image on the metal printing plate. [illegible] these reproducing steps the original spectrum has been [illegible]

1. An aluminum concentration of less than 0.5 p.p.m. is demonstrated by the absence of both aluminum lines, since 0.5 p.p.m. is sufficient to produce line 3961.5.

2. An aluminum concentration of approximately 0.5 p.p.m. and less than 1 p.p.m. is demonstrated by the presence of line 3961.5 in the absence of line 3944.0.

3. The presence of both aluminum lines is evidence of an aluminum concentration of 1 p.p.m. or more.

Biological Distribution of Aluminum.

This spectrographic technique was applied to a number of plant and animal products in order to ascertain the biological distribution of aluminum. The following plant products were examined: wheat germ, yeast, navy beans, Lima beans, potatoes, carrots, and cottonseed meal. The animal products examined were hens' eggs and the following organs and tissues of the rat: liver, kidneys, spleen, testes, ovaries, bone, skeletal muscle, intestinal walls, skin, and lungs. All of these products, with the exception of rat skin, intestinal walls, and lungs, gave spectrograms identical with or at least equivalent to those illustrated by Fig. 1 in that neither aluminum line was present. Rat skin, intestinal walls, and lungs gave spectrograms which contained line 3961.5 but not line 3944.0. The spectrograms of these three products were equivalent to the second spectrogram in Fig. 3, which represents a concentration of 0.5 p.p.m. of aluminum.

This general survey indicates that aluminum is not a constituent of biological material. If present at all its concentration is less than 0.5 p.p.m. because this concentration is sufficient to produce line 3961.5, which was always absent except in the case of rat skin, intestinal walls, and lungs. In these three tissues the aluminum was apparently present but its concentration was less than 1 p.p.m. Otherwise line 3944.0 would have been present. The presence of such a trace of aluminum on the hair, intestinal wall, and in the lungs is to be expected. All of these tissues are always in intimate contact with dust, dirt, and other foreign material in which

its definition. The interpretations and conclusions expressed in this paper are based on examinations of the negatives which show delicate lines more distinctly than do the final half-tones.

aluminum is invariably present. Therefore a trace of aluminum in such tissues is more apt to represent adsorbed foreign matter than any normal tissue constituent.

Dietary Action of Aluminum Compounds.

Young rats were raised to mature ages on diets containing aluminum compounds. The rats so raised were compared with respect to growth, reproduction, and general well being with control rats raised under identical conditions on diets free from

TABLE II.

Composition of Diets.

	Control.	Aluminum chloride.	Baking powder.
Yeast	10.0	10.0	10.0
Casein	20.0	20.0	20.0
Salt Mixture 185*	4.0	4.0	4.0
Agar	2.0	2.0	2.0
Butter fat	8.0	8.0	8.0
Casein	56.0	55.4	53.0
Aluminum chloride		0.6	
Baking powder		0	3.0

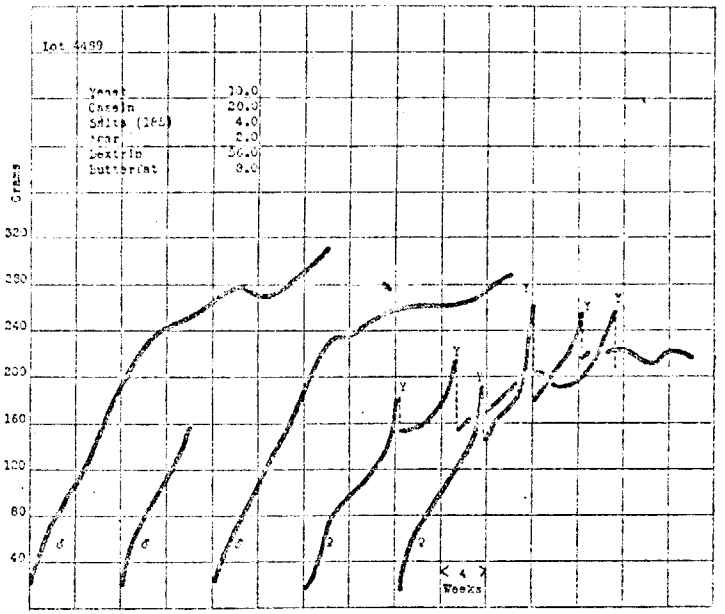
* Composition of Salt Mixture 185.

	gm.
NaCl	146.0
$MgSO_4$ (anhydrous)	225.0
$NaH_2PO_4+H_2O$	293.0
K_2HPO_4	805.0
$CaH_4(PO_4)_2+H_2O$	456.0
Fe citrate (ic)	100.0
Ca lactate	1098.5

aluminum but otherwise identical with the aluminum-containing diets. Two separate and independent experiments were carried out. In one of these the test diet contained 0.6 per cent of aluminum chloride ($Al_2Cl_6 12H_2O$) and in the other the test diet contained 3 per cent of a commercial brand of sodium aluminum sulfate, calcium acid phosphate baking powder, which had been artificially[2]

[2] This artificial decomposition was brought about by mixing with 30 gm. of the fresh powder, 15 ml. of distilled water and heating the resulting mixture at 100° for 30 to 45 minutes in a constant temperature oven.

decomposed or deteriorated previous to incorporation with the other dietary constituents. The composition of the control diet and the two test diets is given in Table II. The baking powder had previously been found by analysis to contain 2.1 per cent aluminum. Accordingly, 0.6 per cent of aluminum chloride and 3 per cent of baking powder represent respectively 0.067 and 0.063 per cent of aluminum in the metallic form. In feeding tests the



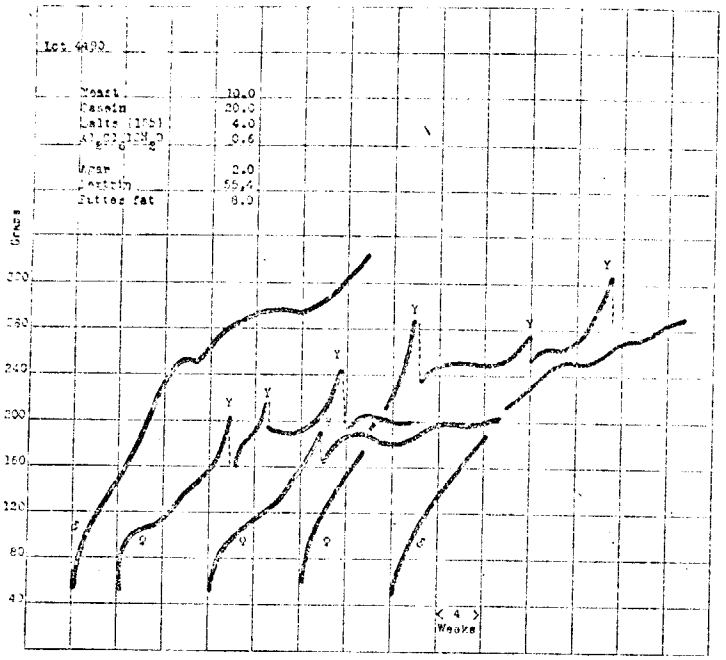
TEXT-FIG. 1. Growth records of control group of rats on aluminum-free diet.

difference between these two concentrations of aluminum may be disregarded.

The rats used in these experiments were born of our breeding stock and weighed 45 to 55 gm. each when started on the diets. Ten of these were placed on the control diet and thereby served as controls. Ten were placed on the aluminum chloride-containing diet, and six on the baking powder-containing diet.

The growth curves of these rats are given on Text-figs. 1, 2, and

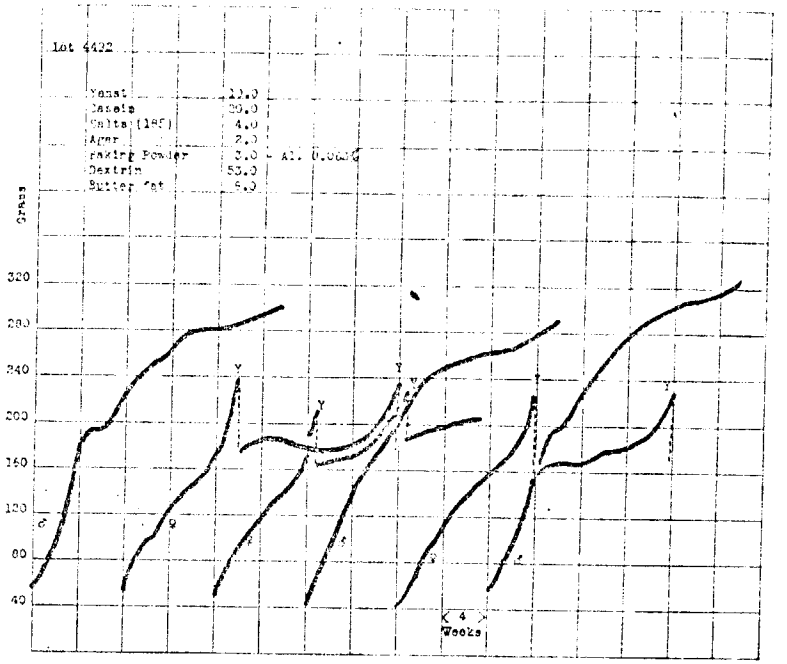
3. These curves also show frequencies of reproduction. Photographs of typical controls and tests are shown by Text-figs. 4 and 5. These charts and photographs show that rats receiving in their diets 600 p.p.m. of metallic aluminum either in form of the chloride or sodium aluminum sulfate, calcium acid phosphate baking powder are the same as the control rats with respect to growth, reproduction, and general appearance. The young of the



TEXT-FIG. 2. Growth records of group of rats fed aluminum chloride-containing diet.

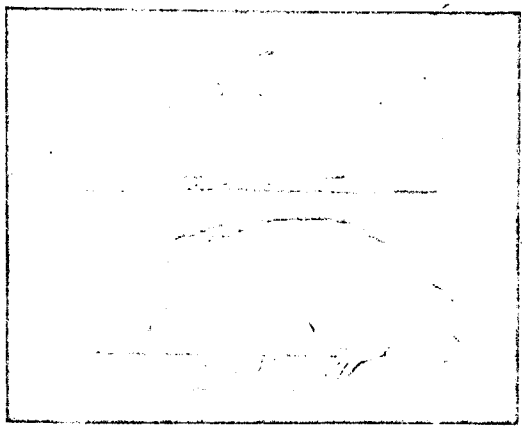
controls were in general discarded but a large number of the young of both of the test groups was examined for the presence of aluminum by the spectrographic technique already described. These examinations were applied to the ashes of the entire bodies of baby rats ranging in age from 1 to 7 days and to the ashes of individual organs of other second generation young after they had been weaned and had subsisted independently for 3 to 4 weeks on

their respective test diets. The ashes of the individual organs examined in this manner were those of liver, kidney, spleen, ovary, testis, skeletal muscle, bone, and intestinal wall. The ashes of the entire bodies of the young baby rats and the ashes of all individual organs except those of the skin, lungs, and intestinal walls gave spectrograms like those illustrated in Fig. 1, indicating the absence of aluminum down to less than 0.5 p.p.m. of the fresh

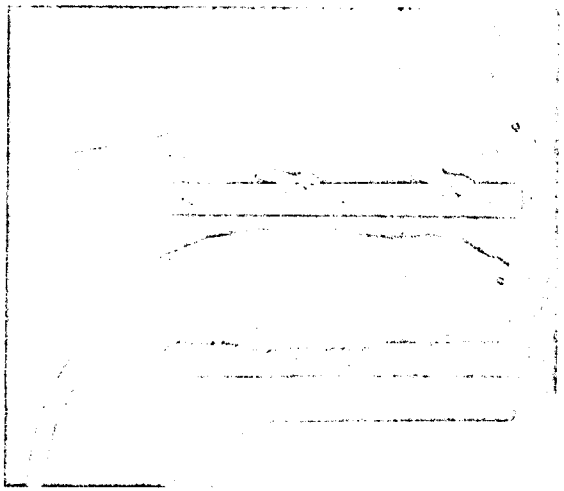


TEXT-FIG. 3. Growth records of group of rats fed aluminum in the form of sodium aluminum sulfate baking powder.

tissue. The spectrograms of the ash of skin contained line 3961.5, but not 3944.0, which indicates a concentration of 0.5 p.p.m. but less than 1 p.p.m. The spectrograms of the ash of intestinal walls contained both lines of such intensity as to indicate very little more than 1 p.p.m. It should be mentioned at this point that the intestinal walls previous to ashing had been slit open and gently agitated for 15 to 20 seconds in each of four successive 1



TEXT-FIG. 4. The rat at the top is the control rat; the lower rat received sodium aluminum sulfate, calcium acid phosphate baking powder.



TEXT-FIG. 5. The rat at the top is the control rat; the lower rat received aluminum chloride.

liter portions of distilled water so that extraneous matter was apparently absent.

The significance of aluminum in the skin, lungs, and intestinal walls has already been discussed. In order to ascertain the persistence with which aluminum in the diet adheres to or is absorbed by the intestinal wall, a series of spectrographic examinations was made of intestinal and stomach walls of rats raised on the three diets; *viz.*, the control diet, the aluminum chloride diet, and the baking powder diet. Typical spectrograms of these experiments are shown by Fig. 4. These spectrograms represent ashes of intestinal and stomach walls of second generation rats which had subsisted on their respective diets 3 to 4 weeks. Previous to ashing each of these alimentary tracts was washed in four successive 1 liter portions of distilled water in the manner already described. The first of these spectrograms is that of the ash of the intestinal tract of a second generation control rat about 2 months old. The second is that of the ash of the intestinal wall of a test rat of the same age which had subsisted on the control diet during the 3 days preceding the experiment. The third is that of the ashes of the intestinal tract of another test rat of the same age which had subsisted on the control diet during the 5 days that preceded the experiment. All spectrograms show the presence of aluminum though less than 1 p.p.m. As might be expected the second spectrogram shows the highest concentration. However, the third shows no more aluminum than does the first, which represents the aluminum content of the intestinal tract of a rat raised on a supposedly aluminum-free diet. Apparently, therefore, the intestinal tract does not absorb or combine permanently with aluminum in the diet, even though the diet contains large quantities, since aluminum which is swept out of the tract in as short a time as 5 days could only have been entrapped in a mechanical manner. In this respect no differences could be detected between aluminum in form of the chloride and in form of sodium aluminum sulfate baking powder residue.

As the experimental work was being terminated, the livers, kidneys, spleens, ovaries, and testes of four of the first generation test rats were examined for the presence of aluminum. At the time of these examinations, these rats had subsisted on the test diets for about 8 months. All of these organs were found to be

aluminum-free or rather to contain less than 0.5 p.p.m. of the element, since their spectrograms were identical with those illustrated by Fig. 1.

During the progress of the feeding experiments, spectrograms were prepared of the ashes of the three diets and of the feces resulting from these diets. These spectrograms are shown by Figs. 5 and 6. It is interesting to note that the control diet appears free from aluminum but that the resulting feces show line 3961.5, indicating something less than 1 p.p.m. of aluminum. Apparently there was a very small trace of aluminum in the test diet, too small to be revealed by the spectrographic method but sufficient to represent 0.5 to 1 p.p.m. of the fecal concentrate.

The heavy aluminum lines of the ash of the aluminum chloride-containing diet are due to 0.1 per cent of metallic aluminum, as compared with 0.033 per cent of metallic aluminum in the baking powder diet. A concentration of 1 per cent of $Al_2Cl_6 12H_2O$ or 0.1 per cent metallic aluminum was used for the first 2 weeks of the feeding experiment. The concentration of the $Al_2Cl_6 12H_2O$ was then lowered to 0.6 per cent in order to equal more nearly the aluminum concentration in the baking powder-containing diet.

The spectrograms in Figs. 5 and 6 indicate, or at least suggest, that any aluminum in the diet is excreted without passing through the walls of the alimentary tract. The spectrograms representing the diet and the feces of the control rats indicate that any concentration of aluminum however small is never absorbed but always excreted.

DISCUSSION OF RESULTS.

As has already been remarked, previous studies of the biological and dietary properties of aluminum compounds have resulted in very diverse views and conclusions. These may be divided into three groups. According to one, aluminum compounds are very toxic and should therefore be excluded from the diet. According to a second group, aluminum is a normal constituent of a large number of both plant and animal tissues; the introduction into the intestinal tract of limited quantities of aluminum is therefore not only a normal and harmless occurrence but is probably even essential to life. According to the third group, to which belong the findings of the Referee Board of Consulting Scientific Experts,

our normal consumption of foods, containing added aluminum, is not deleterious or injurious to health.

The present investigation has yielded results which indicate that the views of the first group are erroneous, and also that the second group is erroneous in its views regarding the wide-spread occurrence of aluminum in biological matter. However, the results of our investigation confirm the views of the Remsen board.

The following are the only conclusions indicated by the present study:

1. Aluminum is not a constituent of either plant or animal matter.

2. Aluminum compounds are not absorbed out of the stomach or intestinal tract when present in the diet.

3. Aluminum compounds when present in the alimentary tract do not form any union or compound with the stomach or intestinal walls.

4. Aluminum compounds in the diet in concentrations as high as 600 p.p.m. of the element aluminum exert no noticeably deleterious action on growth, reproduction, or general well being as judged by external appearance and autopsy.

These conclusions can probably not be regarded as final until after additional and confirming data have been obtained on a larger variety of materials and other animals. But until then there seems to be no other alternative than to accept them as tentative.

This investigation is one of a series on the dietary and other biological properties of supposedly biologically rare elements. A study of certain dietary properties of fluorine has already been reported from this laboratory (10). A consideration of the possible biological functions of manganese is now in progress and in the near future similar considerations will be given to zinc, boron, arsenic, and probably other elements.

BIBLIOGRAPHY.

1. Langworthy, C. F., and Austen, P. T., The occurrence of aluminum in vegetable products and natural waters, New York, 1904.
2. Gonnermann, M., *Biochem. Z.*, 1918, lxxxviii, 401.
3. Bertrand, G., *Bull. Soc. hyg. aliment.*, 1920, viii, 49.

4. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913, xv, 311.
5. Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, lxiii, 143.
6. Gies, W. J., *Biochem. Bull.*, 1916, v, 151.
7. Referee Board of Consulting Scientific Experts of the United States, *U. S. Dept. Agric., Bull. 103*, 1914.
8. Schmidt, C. L. A., and Hoagland, D. R., *Univ. Calif. Pub. Path.*, 1919, ii, 215.
9. de Gramont, A., cited by Twyman, F., Wave length tables, London, 1923.
10. McCollum, E. V., Simmonds, N., Becker, J. E., and Bunting, R. W., *J. Biol. Chem.*, 1925, lxiii, 553.

EXPLANATION OF PLATE 1.

The two short relatively heavy lines at the center of the bottom spectra are the aluminum lines.

FIG. 1. Two typical spectrograms. Top spectrum, empty copper electrodes; middle spectrum, copper electrodes with ash to be tested on lower electrode; bottom spectrum, same as middle spectrum with 1 drop of 0.1 per cent solution of aluminum added.

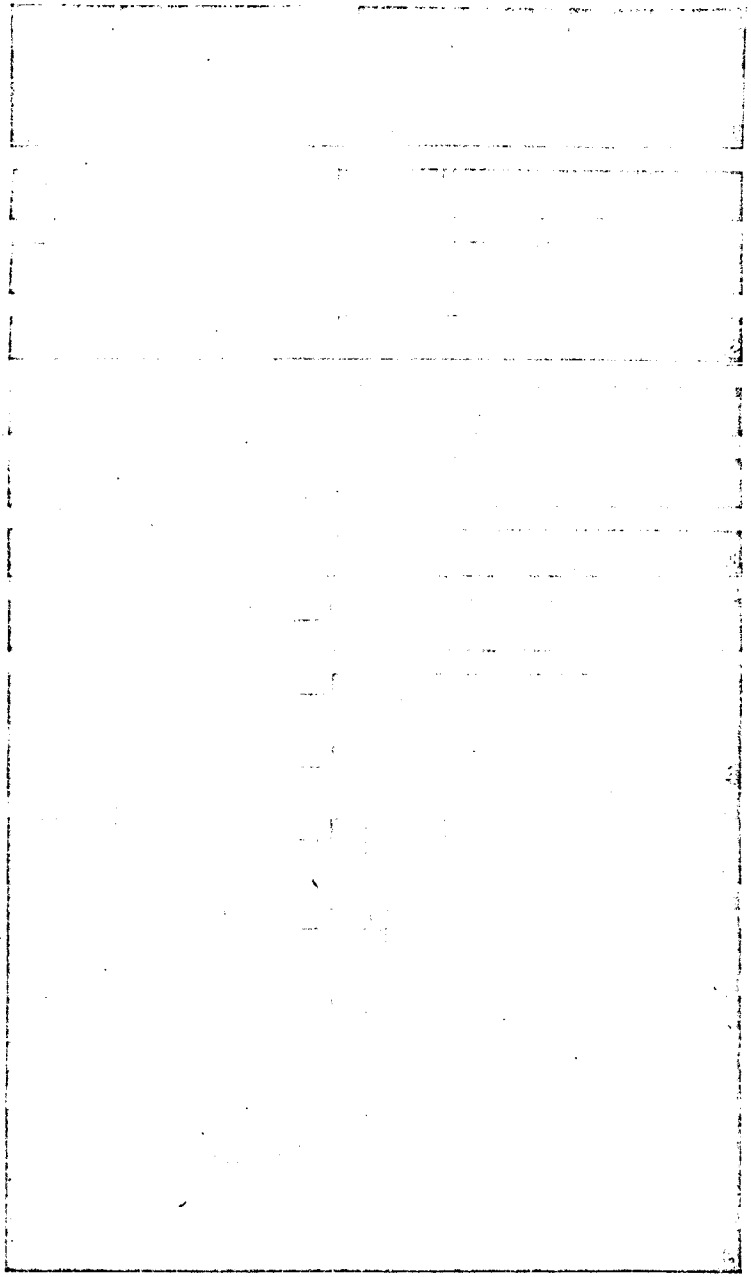
FIG. 2. Spectrogram standards. Top spectrogram, egg ash with no added aluminum; middle spectrogram, egg ash containing aluminum equal to 1 p.p.m. of the fresh egg; bottom spectrogram, egg ash containing aluminum equal to 2 p.p.m. of the fresh egg.

FIG. 3. Spectrogram standards. Top spectrogram, ash of whole egg with no aluminum added; middle spectrogram, ash of whole egg containing an amount of aluminum equal to 0.5 p.p.m. of the fresh egg; bottom spectrogram, ash of whole egg containing an amount of aluminum equal to 1 p.p.m. of the fresh egg.

FIG. 4. Spectrograms of ashes of alimentary tract. Top spectrogram, ash of alimentary tract of a control rat; raiddle spectrogram, ash of alimentary tract of a test rat which had subsisted on the control diet during the 3 days preceding excision of the tract; bottom spectrogram, ash of alimentary tract of a test rat which had subsisted on the control diet during the 5 days preceding excision of the tract.

FIG. 5. Spectrograms of ashes of diets. Top spectrogram, control diet; middle spectrogram, aluminum chloride-containing diet; bottom spectrogram, sodium aluminum sulfate, calcium acid phosphate baking powder-containing diet.

FIG. 6. Spectrograms of ashes of feces. Top spectrogram, feces of control rats; middle spectrogram, feces of aluminum chloride test rats; bottom spectrogram, feces of sodium aluminum sulfate, calcium acid phosphate baking powder test rats.



(McCollum, Rask, and Becker, Aluminum compounds.)

Precision and Accuracy of Colorimetric Procedures as Analytical Control Methods

Determination of Aluminum

5053

ALLEN L. OLSEN, EDWIN A. GEE, AND VERDA McLENDON
Bureau of Mines, Eastern Experiment Station, College Park, Md.

A colorimetric procedure for the determination of aluminum, calculated and represented as aluminum trioxide and involving the formation of the red complex by the interaction of the aluminon reagent and the aluminum ion, has been developed to meet the special requirements in the rapid analysis of leach liquors in pilot-plant operations. The factors influencing color intensities have been investigated and the requisite techniques for a precision and an accuracy of a control character are described. Employing these techniques in the analysis of an aliquot of the leach liquor, precision and accuracy studies as applied to ordinary and refined laboratory techniques have been made on typical analytical data. Statistical reasoning based on the standard deviation is applied to the acquired data. Applying ordinary laboratory techniques, the average precision, measured by the average deviation of the single results from the mean, is of the order of 1% or 10 parts per 1000, while the overall accuracy is of the order of 1 to 3%.

NUMEROUS literature references (*7*, *8*, *12*) and recently published books (*3*, *6*, *17*) describe in detail the procedures involved in colorimetric determinations. Although the colorimetric method has been used for the rapid estimation of small quantities of many inorganic substances, not a great deal of emphasis has been placed on the precision and accuracy that might be expected in its use as an analytical control method. In quality control work, speed is so essential that precision and accuracy are often sacrificed; however, since intelligent conclusions in plant operations have to be based on the analytical data, it is essential to ascertain the precision and accuracy of the control methods.

Recent investigations in this laboratory have been concerned with the colorimetric procedures involving aluminum, titanium, silicon, and sodium. The procedures are, for the most part, adaptations of previously published methods; however, as a matter of convenience, deviations from standard procedures are necessarily made from time to time, and the subsequent effects of the variables on precision and accuracy are briefly considered. Statistical reasoning based on the standard deviation is applied to the acquired data (*1*). The purpose of this investigation is therefore twofold: to describe satisfactory laboratory techniques in colorimetric procedures as applied to aluminum and to evaluate the precision and accuracy that might be expected in routine analyses.

The usual procedure in the colorimetric determination of aluminum involves the formation of the red complex by the interaction of the ammonium salt of aurin tricarboxylic acid (aluminon) and the aluminum ion in a carefully buffered solution (*3*). In the investigation of aluminum in plants, Winter, Thrun, and Bird (*15*) conclude that maximum color is obtained in the presence of 10% ammonium acetate when the solution is maintained at a temperature of 80° C. for 10 minutes and pH 4 (approximately). In the presence of 25 ml. of both ammonium acetate and ammonium chloride, they find that the dye changes color at about pH 7. Roller (*10*) states that the red color which aluminum ion gives with aurin tricarboxylic acid is much more sensitive if made at about pH 6.3 instead of in alkaline solutions as recommended by Yoe and Hill (*16*). The latter authors, investigating the procedure under different experimental conditions, cite five factors that affect the test for aluminum with aluminon: time, temperature, volume, concentration, and the presence of other ions. Lampitt, Sylvester, and Belham (*5*) suggest the use of glycerol to stabilize the lake formed. Thrun (*13*) has investigated the use of protective colloids in colorimetric determination of certain metals as lakes of dyes and recommends the use of a gum arabic solution to keep the aluminum lake of aurin tricarboxylic acid in solution.

The colorimetric method presented here for the determination of aluminum, calculated and represented as aluminum trioxide, has been developed at this station to meet the special requirements in the rapid analysis of leach liquors in pilot-plant operations. The sample taken for analysis must be free from inter-

Ind. Eng. Chem.
16: 169-172 (1944)

Table I. Colorimeter Readings

(0.04 mg. of Al_2O_3)

Test Tube	Reading No. 1	No. 2	Average
1	185	185	185
2	185	185	185
3	185	186	186
4	184	186	185
5[a]	161	166	164
1a[a]	173	168	170
2a[a]	159	157	158
3a[a]	183	178	181
4a[a]	158	158	158
5a[a]	163	163	163
1b	187	186	187
2b	183	186	185
3b	184	187	186
4b	184	187	186
5b	185	187	186

[a] Old test tubes; previous history unknown.

fering ions, which include ferric iron, beryllium, and chromium, since these form a lake similar to that formed by aluminum. Certain variations may be introduced to eliminate these interferences. Chromium lake, for instance, in acetate solution is rapidly decomposed by the addition of ammonia and ammonium carbonate (*6*). Several procedures may be followed for eliminating interfering iron (*4*, *9*). Phosphate, if present in appreciable quantities, prevents the formation of aluminum lakes.

ANALYTICAL PROCEDURE

REAGENTS. *Composite Solution.* Dissolve 154 grams of ammonium acetate, 5 ml. of concentrated hydrochloric acid, 0.400 gram of ammonium salt of aurin tricarboxylic acid and 1 gram of gum arabic in water, and dilute to 1000 ml. Dissolve each reagent in a minimum quantity of distilled water, and add the ingredients of the composite in the order named. The aluminon reagent, weighed out to the nearest milligram, dissolves readily in cold water. To make accurate dilutions, the solution of gum arabic must be cautiously added; otherwise persisting foams will greatly alter the liquid level. The composite solution deteriorates with age, especially when exposed to the light; it therefore, must be protected from light when stored.

Standard Aluminum Solution. Dissolve 4.71 grams of aluminum chloride hexahydrate in 1000 ml. of water (1 ml. = 1 mg. of aluminum trioxide) and standardize gravimetrically (*3*).

Working Standard. Dilute 5 ml. of the standard to 500 ml. (1 ml. = 0.01 mg. of aluminum trioxide).

PROCEDURE. Discharge an aliquot of the previously diluted and acidified leach liquor (10 ml. of liquor and approximately 15 ml. of concentrated hydrochloric acid in 250 ml.), of an amount estimated to contain 0.01 to 0.06 mg. of aluminum trioxide, into a 25-ml. calibrated blood-sugar test tube by means of a pipet, add distilled water to the 12.5-ml. mark, and thoroughly mix the contents. Add 10 ml. of the composite solution by means of an automatic pipet and sufficient water to bring the meniscus to the 25-ml. mark. Mix the contents of the tube well and place in a boiling water bath for precisely 10 minutes. Cool the tube and contents in running tap water for 5 minutes, mix again, and determine the color absorption with the Klett-Summerson photoelectric colorimeter. A filter of range 500 to 570 millimicrons is employed, since a spectrophotometric study of the color in question shows a maximum absorption at 530 mμ in the red complex.

Since absorption of the red color is not a linear function of the aluminum trioxide concentration, a calibration curve must be established. Quantities of the working standard, equivalent to 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg. of aluminum trioxide, are discharged into blood-sugar test tubes, and the lakes are formed in the usual manner.

Procedures for the gravimetric analysis of aluminum involve the use of a modification of the quinolate method (*2*).

From the standpoint of accuracy, ease of manipulation, and rapidity of technique, preliminary investigations indicated that the method of dilution was entirely satisfactory. Calibration of twenty blood-sugar test tubes resulted in an average precision of 0.2% or 2 parts per 1000. No detectable difference in colorimeter reading could be observed in comparing procedures involving pipets, burets, and 25-ml. blood-sugar test tubes.

In an investigation on the factors influencing color intensities, certain anomalous results were obtained in establishing the standardization curve (Table I). Even though these tubes were thoroughly cleaned with chromic acid cleaning solution, it is apparent that only new tubes gave reproducible results. The previous history of the remainder of tubes was unknown. Table II shows the effects of chemically clean test tubes on the reproducibility factor. Tubes in Series I of this table were cleaned with chromic acid cleaning solution; tubes of Series II were cleaned by treating successively with chromic acid, water, ethyl alcohol, benzene, and water; and tubes of Series III were treated with hot chromic acid, water, and alkaline cleaning mixture (*14*) and rinsed thoroughly with distilled water. Thus, to obtain reproducibly accurate results the test tubes must be chemically clean. In all subsequent colorimetric measurements, new tubes only are used, and these are cleaned, using the procedure as established for Series III.

The length of time in the boiling water bath has a marked effect on the color intensity, as shown in Figure 1. The technique of heating at water-boiling temperatures is employed to increase greatly the rate of color development, and since the color intensity varies with the time, the tubes in all of these investigations were heated precisely 10 minutes.

Table II. Cleaning Effects on Blood-Sugar Test Tubes

(0.04 mg. of Al_2O_3)

Test Tube[a]	Colorimeter Readings Series I	Series II	Series III
11	203	196	190
12	192	188	185
13	169	173	185
14	172	178	187
15	175	180	185
11a	163	170	183
12a	187	186	182
13a	188	188	183
14a	167	169	183
15a	169	170	183

[a] Old test tubes; previous history unknown.

The effect of varying quantities of composite on the color intensity is shown in Figure 2. Since the quantity of composite added influences the color intensity, exactly 10 ml. of the aluminon reagent were added from an automatic pipet.

Since the temperature of the sample and reagents is a factor in this method, a control of ±5° C. of the solution temperature at the time of standardization should be maintained. Several degrees above and below that at which the curve is established result in no serious error. High temperatures promote color development, with attendant high aluminas, while lower temperatures have the opposite effect.

In the preparation of the composite solution, quantitative and qualitative techniques were applied to several sources of the re-

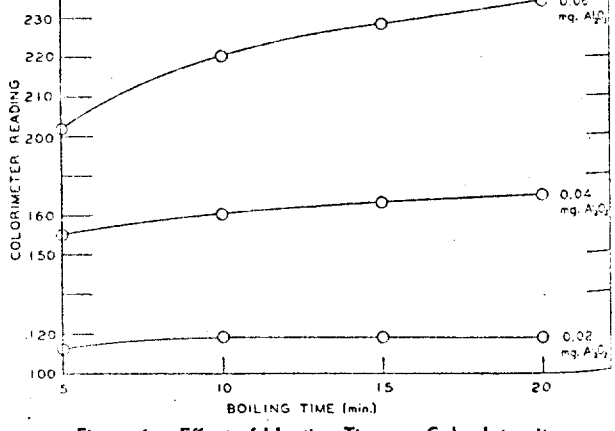


Figure 1. Effect of Heating Time on Color Intensity

agent. To reproduce the values of previously established standardization curves fairly accurately, it became necessary to weigh out the material on a quantitative basis. An Eastman product appeared to give fairly consistent values. Taylor-Austin (*12*), recognizing the variations in the color produced by the method, suggests a restandardization whenever a fresh supply of the solid reagent is put into use. Scherrer and Smith (*11*) state that a satisfactory reagent may be synthesized. However, in view of these variabilities and the fact that the composite undergoes a marked change on standing, even when protected from light, the prime necessity of carrying along a standard in routine analysis cannot be overemphasized. It is common practice in this laboratory to establish a new curve when it is observed that the reading of the standard, when compared with the curve, is in error by an amount greater than 4 or 5%. In the desired concentration range, 0.04 mg. of alumina per 25 ml., this represents 6 or 7 scale divisions. For close control work, especially if the curve has not been determined recently, moderate success has been realized by evaluating the scale divisions in the desired concentration range in terms of milligrams of alumina; thus, by running a standard in the concentration range of the unknown, one can calculate the alumina from the colorimeter reading. Obviously, since the milligrams per unit vary over different parts of the curve as well as for different curves, no permanent values should be assessed to each unit.

PRECISION AND ACCURACY

The precision and accuracy of the colorimetric method are conveniently studied by applying the techniques considered in the foregoing paragraphs to the analysis of a leach solution. An initial investigation was concerned with a factor of reproducibility of results. For this purpose two leach liquors, previously acidified and diluted (10 ml. in 250 ml.) were divided into five portions each, and a 1-ml. aliquot (2 ml. in 250 ml.) of each sample was taken for the measurement. The alumina content, recalculated by applying an appropriate factor for dilution (125), was measured over a period of several weeks. Table III, representing ordinary and refined laboratory techniques, shows what might be expected in the way of precision for 10 typically representative values. The method of evaluating the factor of precision is that derived from consideration of results set up by A.S.T.M. (*1*). As shown in the table, the numerical results are based on the fact that 99 in 100 limits are used and that these results are based completely on the evidence contained in 10 determinations. The accuracy of the method is determined by making a comparison with gravimetric results; gravimetric data for 10 different leach liquors are given in Table IV.

Table III. Analysis of Leach Liquor

Test No.	$Mg./ml.$	d	$(d^2 \times 10^6)$
	Precision of Method under Ordinary Conditions		
1	5.11	−0.063	3969
2	5.23	+0.057	3249
3	5.19	+0.017	289
4	5.23	+0.057	3249
5	5.11	−0.063	3969
6	5.25	+0.077	5929
7	5.14	−0.033	1089
8	5.14	−0.033	1089
9	5.14	−0.033	1089
10	5.19	+0.017	289
Av. = $\bar{X}$	5.173	$\Sigma d^2 = 24210 \times 10^{-6}$	
		$\sqrt{\frac{\Sigma d^2}{10}} = \pm 0.049 = \sigma_{10}$	

$\bar{X} \pm a\sigma = 5.173 \pm 0.053$ ($P_t = 0.99$, 10 observations)
Av. of gravimetric data = 5.114 mg. (Al_2O_3) per ml.

	Precision of Method under Best Conditions		
1	5.11	+0.018	324
2	5.08	−0.012	144
3	5.11	+0.018	324
4	5.04	−0.052	2704
5	5.08	−0.012	144
6	5.08	−0.012	144
7	5.14	+0.048	2304
8	5.07	−0.022	484
9	5.09	−0.002	4
10	5.12	+0.028	784
Av. = $\bar{X}$	5.092	$\Sigma d^2 = 7369 \times 10^{-6}$	
		$\sqrt{\frac{\Sigma d^2}{10}} = \pm 0.027 = \sigma_{10}$	

$\bar{X} \pm a\sigma = 5.092 \pm 0.029$ ($P_t = 0.99$, 10 observations)
Av. of gravimetric data = 5.124 mg. (Al_2O_3) per ml.

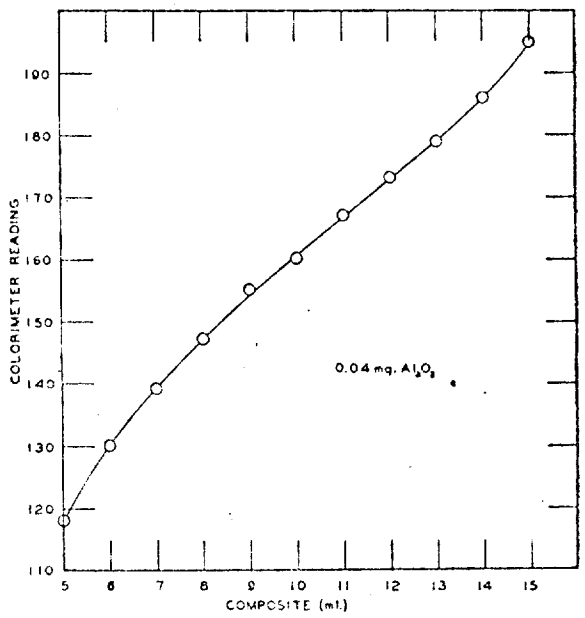


Figure 2. Effect of Varying Quantities of Composite on Color Intensity

Table IV. Analysis of Leach Liquors

Accuracy of method under ordinary conditions

Test No.	Gravimetric $Mg./ml.$	Colorimetric $Mg./ml.$	pH	Error %
1	4.113	4.23	6.0	<3
2	3.468	3.43	6.0	1
3	3.640	3.70	6.0	2
4	3.589	3.63	6.0	>1
5	3.637	3.65	6.0	<1
6	3.926	3.93	...	0
7	3.831	3.97	6.0	3
8	3.855	3.82	6.0	1
9	3.732	3.62	6.0	3
10	3.977	3.97	...	0

DISCUSSION

The expression, $P_t = 0.99$ (statistical probability), cited in Table III implies that a value for a was chosen such that, in 99 chances out of 100, one might expect the ranges bounded by the computed limits to include, of the universe sampled, the objective average, $\bar{X}'$.

From the data in Table III, it is apparent that the colorimetric method should give an average precision, measured by the average deviation of the single results from the mean, of approximately 1%, or 10 parts per 1000. On the basis of the gravimetric value, this represents an accuracy of 1.1%, or 11 parts per 1000. The precision and accuracy are increased by employing refined techniques. In this case special attention was given to temperature control, accurate aliquoting and pipetting, and precise establishment of the standardization curve. However, to attain this precision and accuracy, speed was materially sacrificed. In this case, the average precision becomes 0.6% or 6 parts per 1000. When compared with the gravimetric value, this represents an accuracy of 0.6% or 6 parts per 1000.

The accuracy of the colorimetric method is best judged from the data in Table IV. It is apparent that the accuracy of the method is of the order of 1 to 3%; however, in routine work an occasional 4% error has been observed. In view of the fact that the accuracy is inextricably tied in with the standardization curve, the importance of precise establishment of the calibration curve cannot be overemphasized. If the curve were recently established, then the accuracy and precision become nearly identical if put on the basis of a single analysis. This necessarily follows, since the method involves an empirical comparison against a calibration curve. It is obvious that a higher degree of accuracy will be obtained if, in the preparation of the solution for the colorimetric determination, the concentration of the unknown is approximately adjusted so as to fall in the range above 0.01 mg. per 25 ml.

A further consideration of Table IV reveals the fact that the leach liquors, having been previously acidified with approximately 15 ml. of concentrated hydrochloric acid, when aliquoted to the correct concentration in the presence of excess ammonium acetate, yield a reproducible pH (6.0).

Undoubtedly, colorimetric procedures may be applied for the determination of any element in any given amount by taking suitable aliquot portions for the measurement of the final color; however, applying such a procedure the degree of accuracy will fall markedly as the amount of sample, represented by the aliquots, becomes smaller and smaller. Application of the colorimetric process as a method of analytical control must be decided in terms of the effective range of accuracy by the individual analyst after carefully considering the problem at hand.

ACKNOWLEDGMENT

The authors wish to acknowledge helpful suggestions of J. Conley, chief, Chemical Engineering Unit, Bureau of Mines, the development of the procedure and preparation of this pa

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Manual on Presentation of D... 3rd printing, p. 41, Philadelphia, 1940.
(2) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorg... Analysis", p. 116, New York, John Wiley & Sons, 1929.
(3) Johnson, W. C., *et al.*, Technical Staff, "Organic Reagents ... Metals", London, Hopkin and Williams, 1938.
(4) Kul'berg, L. M., and Rovinskaya, D. I., *Zavodskaya Lab.*, 9 (1940).
(5) Lampitt, L. H., Sylvester, N. D., and Belham, P., *Analyst*, 57, 418 (1932).
(6) Mellan, I., "Organic Reagents in Inorganic Analysis", Philadelphia, Blakiston Co., 1941.
(7) Mellon, M. G., Ind. Eng. Chem., Anal. Ed., **11**, 80 (1939).
(8) Muller, R. H., *Ibid.*, **11**, 1 (1939).
(9) Musakin, A. P., *Zavodskaya Lab.*, 9, 567 (1940).
(10) Roller, P. S., *J. Am. Chem. Soc.*, 55, 2437 (1933).
(11) Scherrer, J. A., and Smith, W. H., *J. Research Natl. Bur. Standards*, **21**, 113 (1938).
(12) Taylor-Austin, E., *J. Soc. Chem. Ind.*, 60, 29 (1941).
(13) Thrun, W. E., Ind. Eng. Chem., Anal. Ed., **2**, 8 (1930).
(14) Willard, H. H., and Furman, N. H., "Elementary Quantitative Analysis", 3rd ed., p. 15, New York, D. Van Nostrand Co., Inc.
(15) Winter, O. B., Thrun, W. E., and Bird, O. D., Ind. Eng. Chem., Anal. Ed., 51, 2721 (1929).
(16) Yoe, J. H., and Hill, W. L., *J. Am. Chem. Soc.*, 49, 2395 (1927).
(17) Yoe, J. H., and Sarver, L. A., "Organic Analytical Reagents", New York, John Wiley & Sons, 1941.

Published by permission of the Director, U. S. Bureau of Mines, Washington, D. C.

J. Am. Chem Soc. 55: 2437-38 (1933) 5051

Colorimetric Determination of Aluminum with Aurintricarboxylic Acid[1]

BY PAUL S. ROLLER[2]

The aurintricarboxylic test of Hammett and Sottery[3] has been shown by Yoe and Hill[4] to be adaptable to the quantitative estimation of aluminum. In the recommended quantitative procedure, as directed for the qualitative test, the reaction mixture is made alkaline with NH_4OH–$(NH_4)_2CO_3$ before colorimetric comparison. The resultant gradual fading and the evolution of bubbles have been found troublesome.[5] By comparison with a slightly acid solution, it is found, as shown below, that the alkaline treatment also greatly reduces the sensitivity. The alkaline solution masks the effect of Cr^{+++}, but since this effect is relatively slight, and since the manner of its complete elimination is indicated, it is proposed that in general the colorimetric determination of aluminum with aurintricarboxylic acid be carried out at a fixed acid P_H.

The yellow color of the dye in alkaline solution and its red color in acid solution practically neutralize each other at a P_H of 6.3 in buffered solution. Hence a P_H of 6.3 was adopted as the fixed acid P_H at which comparison is made.

In the present procedure, adapted to colorimeter comparison, X cc. of the aluminum solution at about P_H 6.3 is diluted with $(12-X)$ cc. of water, and 5 cc. of a buffer (P_H 6.3) is added. The buffer is 4 M ammonium acetate containing some hydrochloric acid. After shaking, 1 cc. (per 0.01 mg. of aluminum) of a 0.1% solution of the ammonium salt of the dye is added and the mixture is again thoroughly shaken. The maximum color intensity is reached in about fifteen minutes. The color is stable over a period of many hours. As in alkaline solution,[5,6] the measured intensity is not linearly proportional to the quantity of aluminum. For amounts of aluminum less than 0.002 mg., comparison must be made in Nessler tubes.

The non-interference of other elements originally observed is found to obtain also under the present conditions. A blank result was given by the following: 10 mg. of Ba^{++}, Ca^{++}, Mg^{++}, Zn^{++}, Pb^{++}; 0.1 mg. of Co^{++}, Cu^{++}; 5 mg. of $PO_4^{\equiv}$. One mg. of SiO_2 from a solution of a crystallized silicate gave a color equivalent to 0.001 mg. of aluminum, no doubt due to an impurity of aluminum.

As is well known, Fe^{+++} is a major source of interference and must be eliminated. Measured in a colorimeter, 0.010 mg. of Fe gave a color equivalent to 0.005 mg. of aluminum.

(1) Published by permission of the Director, U. S. Bureau of Mines. (Not subject to copyright.)

(2) Associate chemist, U. S. Bureau of Mines, Nonmetallic Minerals Experiment Station, Rutgers University, New Brunswick, N. J.

(3) Hammett and Sottery, THIS JOURNAL, 47, 142 (1925).

(4) Yoe and Hill, *ibid.*, 49, 2295 (1927).

(5) Myers, Mull and Morrison, *J. Biol. Chem.*, 78, 595 (1928); Schwartze and Hann, *Science*, 69, 169 (1929).

(6) Winter, Thrun and Bird, THIS JOURNAL, 51, 2721 (1929).

Cr^{+++} in chrome alum solution was found to react slowly with the dye. The green Cr^{+++} reacts more rapidly than the blue; increase of temperature hastens the reaction. At room temperature, 0.10 mg. of green Cr^{+++} gave after fifteen minutes a color equivalent to 0.0005 mg. of aluminum, after thirty minutes 0.001 mg. of aluminum, and after eighteen hours 0.008 mg. of aluminum. It is seen that Cr^{+++} is but a slight source of interference under the conditions. Complete elimination of this interference is indicated by an increase in P_H dependent on the quantity of Cr^{+++} with, of course, a resultant decrease in over-all sensitivity of the test.

Under the present conditions, the aurin reaction with aluminum is extremely delicate. A faint pink is obtained with 0.0001 mg. of aluminum, so that the sensitivity is about twenty times that reported by Yoe and Hill under alkaline conditions. Besides the elimination of fading and of carbon dioxide evolution, turbidity in the presence of foreign ions such as that observed by Yoe and Hill is also obviated.

NEW BRUNSWICK, NEW JERSEY

RECEIVED MARCH 1, 1933
PUBLISHED JUNE 6, 1933

DRUGS

Infrared Spectra of Some Compounds of Pharmaceutical Interest

By OSCAR R. SAMMUL, WILSON L. BRANNON, and ALMA L. HAYDEN (Division of Pharmaceutical Chemistry, Food and Drug Administration, Washington, D.C. 20204)

This compilation of infrared absorption spectra is intended as a supplement to the spectra of the USP and NF Reference Standards published in *This Journal*, 45, 797 (1962). This second group is composed of new and nonofficial drugs, USP and NF items, solvents, and reagents.

In general the samples were prepared and the spectra were recorded by the techniques and under the conditions which were described in the earlier publication. Most of the solids were observed in potassium halide disks after recrystallization from various solvent systems. The spectra of some compounds were obtained in carbon disulfide, tetrachloroethylene, and chloroform solutions in 1 mm NaCl cells. Other compounds were studied as films, paraffin oil or hexachlorobutadiene mulls, and vapors. The spectra of the vapors were obtained in a 10 cm gas cell, at room temperature and reduced pressure.

The spectra are arranged in general alphabetical order and the experimental conditions, namely, crystallization solvent(s) and infrared medium, are given in Table 1. These spectra and those included in the earlier publication have been incorporated in the Termatrex System for data storage and retrieval (Jonkers Business Machines Corporation). Numbers following the conditions for each compound in the table are Termatrex code numbers.

Acknowledgments

The technical assistance of Stephen R. Seater, J. Donald Weber, Alden H. Reine, and Ruth Kessler, and the editorial assistance of Marie C. Talley and Helen L. Reynolds are greatly appreciated.

The authors gratefully acknowledge the compounds and spectra which were contributed by Millard Maienthal, Llewellyn H. Welsh, and others of this Division.

Table 1. Drugs and their spectral conditions

Name	No.	Conditions	Retrieval No.	Page No.
Acacia	208	Direct KBr	0032	925
Acetic Acid	209	Film	1042	925
Acetone	213	Vapor	1031	926
Acetophenazine Dimaleate	211	EtOH; KBr	8200	926
Acetophenetidine	210	$CHCl_3$; KBr	4087	925
N-Acetyl-*d*-amphetamine	212	C_6H_{14}-petr ether; KBr	8233	925
N-Acetyl-*dl*-amphetamine	214	C_6H_{14}-petr ether; KBr	8232	925
Acetyl Salicylic Acid	215	Direct KBr	3018	926
N^4-Acetylsulfanilamide	216	Me_2CO; KBr	4062	926
Akineton Hydrochloride	217	Direct KCl	8108	926
Aluminum Acetate	218	Direct KBr	1020	927
Alvodine Base	219	Direct KBr	8107	927
dl-Amidon Hydrochloride	220	$CHCl_3$; KCl	4061	927
Aminoacetic Acid	228	EtOH-H_2O; KBr	2051	929
2-Amino-5-chlorobenzophenone	222	Direct KBr	4070	927
4-Amino-6-chloro-*m*-benzenedisulfonamide	221	Direct KBr	4081	927
Aminophylline	223	Direct KBr	8137	928
p-Aminosalicylic Acid	224	H_2O; KBr	4059	928

(Continued)

Name
Ammonium Chloride
Amobarbital
d-Amphetamine Hydrochloride
n-Amyl Alcohol
n-Amyl Alcohol
Amylene Hydrate
Amyl Metacresol
delta-5-Androstene-3-beta-17-beta-diol
delta-5-Androstene-3-beta-ol-17-one
delta-4-Androstene-3,17-dione
delta-4-Androstene-3,17-dione
Angiotensin Amide
Anhalamine Hydrochloride
Anhalonine Hydrochloride
Ansolysen Bitartrate
Anthracene
Anthralin
Anthrone
Antipyrine
Apomorphine Hydrochloride
Aprobarbital
Azaphenothiazine
Aspirin Anhydride
Atropine
Barbital
Bentyl Analog
Benzaldehyde
Benzoic Acid
Benzoic Acid
Benzoin
Benzphetamine Hydrochloride
Benzthiazide
Benztropine Methanesulfonate
Benzyl Alcohol, Redistilled
Benzyl Alcohol, Redistilled
Betamethasone
Betazole Hydrochloride
d-Brompheniramine Maleate
Bunamiodyl
Butabarbital
Butethamine Hydrochloride
n-Butyl Alcohol
sec-Butyl Alcohol
Butylated Hydroxytoluene
Caffeine
Calcium Cyanamide
Camphor
Camphor-10-sulfonic Acid
Caramiphen Ethanedisulfonate
Carbimazole
Carbocaine Hydrochloride
Carbon Disulfide
Cardrase
Castor Oil
Cellulose
Chlordantoin
Chlorhexidine Dihydrochloride
Chloroprocaine Hydrochloride
Chlorpropamide
Cholesterol

OF THE A.O.A.C. (Vol. 47, No. 5, 1964)

s of Pharmaceutical

and ALMA L. HAYDEN (Division
istration, Washington, D.C. 20204)

ectra are arranged in general alpha-
rder and the experimental condi-
namely, crystallization solvent(s) and
medium, are given in Table 1.
pectra and those included in the
ublication have been incorporated
Termatrex System for data storage
eval (Jonkers Business Machines
on). Numbers following the condi-
or each compound in the table are
rex code numbers.

Acknowledgments

technical assistance of Stephen R.
Donald Weber, Alden H. Reine,
Kessler, and the editorial assist-
Marie C. Talley and Helen L. Rey-
re greatly appreciated.

gratefully acknowledge the
spectra which were contrib-
y Minard Malcuthal, Llewellyn H.
and others of this Division.

... conditions

...itions	Retrieval No.	Page No.
KBr	0032	925
	1012	925
	1031	926
l Br	8200	926
Br	4087	925
r ether; KBr	8233	925
r ether; KBr	8232	925
	3018	926
Br	4062	926
l	8108	926
Br	1020	927
Br	8107	927
Cl	4061	927
; KBr	2051	929
	4070	927
Br	4084	927
	8137	928
	4059	928

(Continued)

Table 1. (*Continued*)

Name	No.	Conditions	Retrieval No.	Page No.
Ammonium Chloride	225	Direct KCl	0014	928
Amobarbital	229	C_5H_5; KBr	8230	929
-Amphetamine Hydrochloride	226	Direct KBr	4094	928
-Amyl Alcohol	230	Film	1041	929
-Amyl Alcohol	231	CS_2 solution	1052	929
Amylene Hydrate	227	Vapor	1026	928
Amyl Metacresol	232	Film	3021	929
elta-5-Androstene-3-beta-17-beta-diol	234	Direct KBr	6055	930
elta-5-Androstene-3-beta-ol-17-one	236	CS_2 solution	6054	930
lta-4-Androstene-3,17-dione	237	CS_2 solution	6052	930
elta-4-Androstene-3,17-dione	235	Direct KBr	6053	930
Angiotensin Amide	238	Direct KBr	8213	931
Anhalamine Hydrochloride	239	Direct KCl	7020	931
Anhalonine Hydrochloride	240	Direct KCl	8124	931
Ansolysen Bitartrate	242	Direct KBr	8106	931
Anthracene	241	Direct KBr	3051	931
Anthralin	243	$CHCl_3$ solution	3048	932
Anthrone	233	$CHCl_3$-C_7H_{16} (1:3); KBr	3052	930
Antipyrine	244	EtOH-C_7H_{16} (1:3); KBr	8138	932
Apomorphine Hydrochloride	245	Direct KCl	8139	932
Aprobarbital	246	EtOH-H_2O (1:1); KBr	8182	932
Azaphenothiazine	249	EtOH-C_7H_{16} (1:3); KCl	8189	933
Aspirin Anhydride	247	EtOH-H_2O (95:5); KBr	3019	932
Atropine	248	Direct KBr	7012	933
Barbital	250	H_2O; KBr	8195	933
Beatyl Analog	251	EtOH-C_7H_{16} (1:3); KCl	2041	933
Benzaldehyde	253	Film	3013	934
Benzoic Acid	252	CS_2 solution	3023	933
Benzoic Acid	254	Direct KBr	3038	934
Benzoin	255	H_2O-Me_2CO; KBr	3039	934
Benzphetamine Hydrochloride	258	Direct KCl	4073	935
Benzthiazide	259	Direct KBr	8172	935
Benztropine Methanesulfonate	260	Direct KBr	8196	935
Benzyl Alcohol, Redistilled	256	CS_2, C_2Cl_4 solutions	3045	934
Benzyl Alcohol, Redistilled	257	Film	3044	934
Betamethasone	261	Direct KBr	6071	935
Betazole Hydrochloride	262	Mull	8167	935
-Brompheniramine Maleate	263	Direct KBr	8104	936
Bunamiodyl	264	EtOH-C_7H_{16} (1:3); KBr	4066	936
Butabarbital	265	CH_3OH-C_7H_{16} (1:3); KBr	8212	936
Butethamine Hydrochloride	266	EtOH; KCl	4063	936
-Butyl Alcohol	267	Vapor	1027	936
sec-Butyl Alcohol	268	Vapor	1028	937
Butylated Hydroxytoluene	269	CS_2, C_2Cl_4 solutions	3030	937
Caffeine	270	Direct KBr	8087	937
Calcium Cyanamide	271	Direct KBr	0010	937
Camphor	272	CS_2, C_2Cl_4 solutions	1036	937
Camphor-10-sulfonic Acid	273	EtOAc; KBr	2019	938
Caramiphen Ethanedisulfonate	274	Me_2CO-C_7H_{16} (1:1); KBr	2021	938
Carbinazole	275	EtOH; KBr	8102	938
Carbocaine Hydrochloride	276	Direct KCl	8155	938
Carbon Disulfide	292	Vapor	2055	941
Cardrase	277	Me_2CO-C_7H_{16} (1:3); KBr	8108	938
Castor Oil	278	Film	0023	939
Cellulose	279	Direct KBr	1010	939
Chlordantoin	280	CS_2, C_2Cl_4 solutions	8204	939
Chlorhexidine Dihydrochloride	281	Direct KCl	4042	939
Chloroprocaine Hydrochloride	282	EtOH-C_7H_{16} (1:3); KCl	4041	939
Chlorpropamide	283	EtOH-C_7H_{16} (1:3); KBr	4037	940
Cholesterol	284	CS_2-C_2Cl_4 solutions	6076	940

(Continued)

Table 1. *(Continued)*

Name	No.	Conditions	Retrieval No.	Page No.
Cholesterol	285	Et_2O; KBr	6072	940
Cinchonidine	288	C_6H_6; KBr	7035	941
Cinchonine	289	C_6H_6; KBr	7036	941
Citric Acid	286	C_6H_5OH; KBr	1023	940
Codeine	287	CS_2, C_2Cl_4 solutions	7027	940
Codeine	293	CS_2; KBr	7014	942
Codeine Phosphate	291	EtOH-H_2O (9:1); KBr	7028	941
Corn Oil	290	Film	0035	941
Corn Oil	291	Film	3040	941
Cyclothiazide	295	EtOH-C_6H_{12} (1:3); KBr	8205	942
Cyproheptadine Hydrochloride	296	Me_2CO-C_6H_{12} (1:3); KCl	8109	942
Dequadin Chloride	297	CH_3OH-C_6H_{12}-C_3H_7OH (0.5:0.5:3.0); KCl	8113	942
Dequalinium Chloride	298	Direct KCl	8110	942
Desoxycorticosterone Acetate	299	CS_2 solution; $CHCl_3$	6032	945
Desoxycorticosterone Trimethylacetate	300	CS_2, C_2Cl_4 solutions	6077	943
Desoxyephedrine Hydrochloride	301	Direct KCl	4060	945
Dexamethasone	302	Me_2CO-isoC_5H_{12} (1:3); KBr	6038	942
Dexamethasone Acetate	303	Me_2CO-isoC_5H_{12} (1:3); KBr	6036	941
Dextrochlorpheniramine Maleate	304	Direct KBr	8009	944
Dextrose, Anhydrous	306	Direct KBr	1011	941
Diacetylchondrocurarine Iodide	305	H_2O with added KI) KI	7038	941
N,O-Diacetylphenylephrine	313	C_6H_6; KBr	4093	946
N,O-Diacetyl-*p*-hydroxyamphetamine	307	CCl_4; KBr	4057	944
Dibenzothiophene	308	EtOH-C_6H_{12} (1:3); KBr	8210	942
Dihydrochlorothiazide	309	EtOH-C_6H_{12} (1:3); KBr	8118	943
1,8-Dihydroxyanthraquinone	311	$CHCl_3$ solution	3049	946
Dilabron Methanesulfonate	310	EtOH-MeOH-isoC_5H_{12} (1:1:5); KBr	4013	945
Dimethoxinate Hydrochloride	312	Direct KCl	8112	945
p,p-Dimethoxydiphenylacetylene	315	Glacial CH_3COOH; KBr	3050	946
Dimethpyrindene Maleate	318	Direct KBr	8174	947
Dimethylaminopropyl-*d*-camphidine Dimethyl Sulfate	311	Direct KBr	7021	945
3,5-Dimethyl-2-nitroanisole	316	Film	4095	946
3,5-Dimethyl-4-nitroanisole	317	EtOH-H_2O; KBr	4101	946
Diphenylhydantoin	319	Direct KBr	8148	947
Diphenylpyraline Hydrochloride	320	Direct KCl	8100	947
Dromostanolone Propionate	321	EtOH-isoC_5H_{12} (1:9); KBr	6066	947
Dynacaine Hydrochloride	322	Direct KCl	7025	947
Ectylurea	323	$CHCl_3$-C_6H_{12} (2:3); KBr	2024	948
Enovid	324	CS_2 solution	6057	948
Enovid	325	EtOH-C_6H_{12} (1:3); KBr	6056	948
Ephedrine Sulfate	326	C_3H_7OH-Et_2O-H_2O-isoC_5H_{12} (4.5:5.0:0.5:40.0); KBr	7011	948
Equilenin	328	EtOH; KBr	6059	949
Equilin	327	EtOH; KBr	6058	948
Erythritol Tetranitrate	329	$CHCl_3$; KBr	2032	949
Erythromycin Propionate	330	Et_2O-isoC_5H_{12} (0.3:3.7); KBr	2022	949
beta-Estradiol	331	Direct KBr	6060	949
Estradiol Dipropionate	332	CS_2, C_2Cl_4 solutions	6079	949
Ethanol	333	Vapor	1022	950
Ether	334	Vapor	1025	950
Ethinyl Estradiol-3-benzoate	335	MeOH; KBr	6062	950
Ethinyl Nortestosterone	336	EtOH-C_6H_{12} (1:3); KBr	6037	950
Ethyl Nitrate	337	CS_2 solution	2038	950
2-Ethyl Thioisonicotinamide	338	EtOH-C_6H_{12} (1:7); KBr	8176	951
Ethynyl Estradiol-3-methyl ether	339	EtOH-C_6H_{12} (1:5); KBr	6035	951

(Continued)

(Continued)

	Retrieval No.	Page No.
Br	6072	949
KBr	7035	949
KBr	7036	941
KBr	1023	946
solutions	7027	946
r	7014	942
H_2O (9:1); KBr	7028	942
	0035	941
	3040	941
H_{16} (1:3); KBr	8205	942
H_{16} (1:3); KCl	8109	942
H-C_5H_{16}-C_2H_5OH (3.0); KCl	8113	942
	8110	943
on; $CHCl_3$	6032	943
Cl_3 solutions	6077	943
	4069	943
C_5H_{16} (1:3); KBr	6038	943
C_5H_{16} (1:3); KBr	6036	941
KBr	8039	941
KBr	1014	941
added KI) KI	7038	941
r	4093	946
KBr	4057	941
C_5H_{16} (1:3); KBr	8210	945
C_5H_{16} (1:3); KBr	8118	945
tion	3049	946
OH-H_{16} (1:1:5); KBr	4043	945
KCl	8112	945
; KBr	3050	946
	8174	947
KBr	7024	945
	4095	946
; KBr	4101	946
	8148	947
K	8100	947
C_5H_{16} (1:9); KBr	6066	947
KCl	7025	947
(2:3); KBr	2024	948
tion	6057	948
H_{16} (1:3); KBr	6056	948
H_2O-H_2O- 1.5:5.0:0.5:40.0);		948
	7011	
KBr	6059	949
KBr	6058	948
KBr	2032	949
(0.3:3.7); KBr	2022	949
	6060	949
solutions	6079	949
	1022	950
	1025	950
r	6062	950
(1:3); KBr	6037	950
tion	2038	950
H_{16} (1:7); KBr	8176	951
H_{16} (1:5); KBr	6035	951

Table 1. *(Continued)*

Name	No.	Conditions	Retrieval No.	Page No.
Cyclamine Acetate	340	EtOH-C_5H_{16} (1:3); KBr	8175	951
Alcohol	341	Film	3025	951
Cinic Sodium Ethylenediamine Tetraacetate	342	Direct KBr	2026	951
Diacetonide Acetonide	343	Direct KBr	6068	952
5-Fluorouracil	344	Direct KBr	8165	952
Fluoxymesterone	345	EtOAc-C_5H_{16} (1:1); KBr	6034	952
Fluphenazine Dihydrochloride	346	EtOH-C_5H_{16} (1:3); KCl	8236	952
Fluphenazine Dihydrochloride	347	Direct KCl	8157	952
Fluprandrenolone	348	$CHCl_3$-C_5H_{16} (1:7); KBr	6075	953
Fluracetamide	349	Film	2030	953
Fluritanol	350	Direct KBr	8178	953
Fluorogenin	351	EtOH; KBr	6063	953
Fluorosteryl Acetate	352	Film	1009	953
Glycopyrrolate	353	Direct KBr	8199	954
Griseofulvin	354	EtOH-C_5H_{16} (1:3); KBr	8119	954
Guaiacol	355	Film	3026	954
1,3-Hexachlorobutadiene	356	Film	1038	954
Hexachlorophene	357	CS_2, C_2Cl_4 solutions	3032	954
Hexadimethrine Bromide	358	Direct KBr	2023	955
Hexestrol	359	Direct KBr	3022	955
Hexetidine	360	Film	8116	955
Hexocyclium	361	$CHCl_3$-C_5H_{16} (1:3); KBr	8209	955
Hexylethyl Barbituric Acid	362	Direct KBr	8119	955
Hydrocortamate	363	Direct KBr	6039	956
Hydrocortisone-21-phosphate Disodium Salt	365	Direct KBr	6040	956
Hydroflumethiazide	366	Direct KBr	8117	956
17-Hydroxydesoxycorticosterone	367	Direct KBr	6061	956
3-Hydroxymercuri-2-methoxysuccinamido-propane Theophyllinate	368	Direct KBr	9501	957
2-Hydroxy-2-phenylethylcarbamate	369	EtOH-isoC_5H_{16} (1:10); KBr	4049	957
Hyoscine Hydrobromide	363	Direct KBr	7018	956
Iodate Calcium	371	Direct KBr	4071	957
Iodate Sodium	372	Direct KBr	4072	957
Iso-octane	370	Film	1053	957
Isopregenone	373	Direct KBr	6073	958
Isopropamide Iodide	374	C_5H_{16}-MeOH-isoC_5H_{16} (1:1:10); KI	4041	958
Isosorbide Dinitrate	375	Et_2O; KBr	2037	958
Isuprel Ethanesulfonate	376	Direct KBr	4046	958
Kanamycin Sulfate	377	Direct KBr	2027	958
Lactose	378	Direct KBr	1013	959
Levarterenol Bitartrate	379	Direct KBr	4015	959
Librium base	380	CS_2, C_2Cl_4 solutions	8169	959
Librium Hydrochloride	381	Direct KCl	8170	959
Linoleic Acid	383	Film	1050	960
Lophophorine Hydrochloride	382	Direct KCl	7019	959
Mannitol Hexanitrate	384	Direct KBr	2034	960
Menadione	385	CS_2, C_2Cl_4 solutions	3047	960
Menthol	386	$CHCl_3$ solution	1006	960
Mephenoxalone	387	Me_2CO-C_5H_{11} (1:3); KBr	8171	960
Meprobamate	388	H_2O; KBr	2031	961

(Continued)

Table 1. (*Continued*)

Name	No.	Conditions	Retrieval No.	Pa. N
Mescaline Hydrochloride	389	Direct KCl	4039	961
Mescaline Sulfate	390	Direct KBr	7010	961
Meta-Butethamine Hydrochloride	409	EtOH; KCl	4064	962
Metaxalone	391	Me_2CO; KBr	8177	961
Methalamic Acid	392	$isoC_5H_{11}OH$-C_7H_{16} (1:3); KBr	4076	963
Methanol	393	Vapor	1032	962
Methaqualone	394	Direct KBr	8198	962
Metharbital	395	EtOH; KBr	8039	962
Methenamine Mandelate	396	Direct KBr	8129	962
Methenamine Undecylenate	397	Melt	8091	962
Methocarbamol	398	EtOH-C_7H_{16} (0.4:0.6); C_7H_{16} (1:5); KBr	4017	963
Methoxyflurane	399	Film	1035	963
p-Methoxythiobenzaldehyde	410	Direct KBr	4096	962
Methsuximide	407	EtOH; KBr	8231	961
Methyl Acetate	400	Vapor	1033	963
6-alpha-Methyl-17-acetoxy-progesterone	401	Direct KBr	6041	963
Methyldopate Hydrochloride	411	Direct KCl	4103	967
6-Methylhydrocortisone Acetate	402	EtOH-$isoC_5H_{11}$-$isoC_8H_{18}$ (0.2:0.5:5.0); KBr	6082	963
3-Methyl-4-nitroanisole	412	EtOH-H_2O; KBr	4097	963
3-Methyl-3-pentanol Carbamate	403	EtOH (30%); KBr	2025	963
6-Methyl-prednisolone	404	nC_4H_9OH-$isoC_5H_{11}$-$isoC_8H_{18}$ (0.2:0.8:5.0); KBr	6042	963
Methyl Testosterone	405	CS_2, CCl_4 solutions	6047	963
Metopirone	406	Direct KBr	8179	963
Mikedimide	408	EtOH-$isoC_5H_{11}$-$isoC_8H_{18}$ (0.2:0.8:5.0); KBr	8128	963
Neomycin Undecylenate	414	$CHCl_3$-C_7H_{16} (1:3); KBr	0020	966
Niakamide	415	Direct KBr	8130	963
Nicotinic Acid	416	Mull	8168	966
p-Nitrofluorobenzene	413	Film	1100	966
Nitroglycerine	417	CS_2 solution	2033	966
Nitromethane	418	Vapor	2015	967
19-Nor-delta-4-androstene-17-beta-ol-3-one-beta-phenylpropionate	419	Direct KBr	6045	967
Norethisterone Acetate	420	EtOH-$isoC_8H_{18}$ (1:10); KBr	6044	967
Nostal	421	EtOH-H_2O; KBr	8184	967
Olive Oil	422	Film	0038	967
Orphenadrine Citrate	423	Direct KBr	4050	968
Orphenadrine Hydrochloride	424	Direct KCl	4048	968
Oxyphencyclimine Hydrochloride	426	EtOH-C_7H_{16} (1:3); KBr	8154	968
Oxytetracycline	425	Mull	4069	968
Palmitic Acid	427	CS_2 solution	1045	968
Palmitic Acid	428	CS_2; KBr	1044	969
Parabromdylamine Maleate	429	Direct KBr	8089	969
Paraffin Oil	430	Film	0025	969
Peanut Oil	483	Film	0033	980
Pentaerythritol Tetranitrate	432	EtOH-Me_2CO; KBr	2035	969
Pentamethylene Tetrazole	431	Direct KBr	8153	969
Pentobarbital	433	C_6H_{12}-C_7H_{16}; KBr	8152	970
Pentobarbital	434	CCl_4 solution	8147	970
Perphenazine	435	Direct KBr	8090	970
Persantin	436	$isoC_5H_{11}OH$-C_7H_{16} (1:4); KBr	8097	970
Phenazocine Hydrobromide	437	Direct KBr	8125	970
Phendimetrazine Bitartrate	438	$isoC_5H_{11}OH$-C_7H_{16} (1:3); KBr	8201	971

(*Continued*)

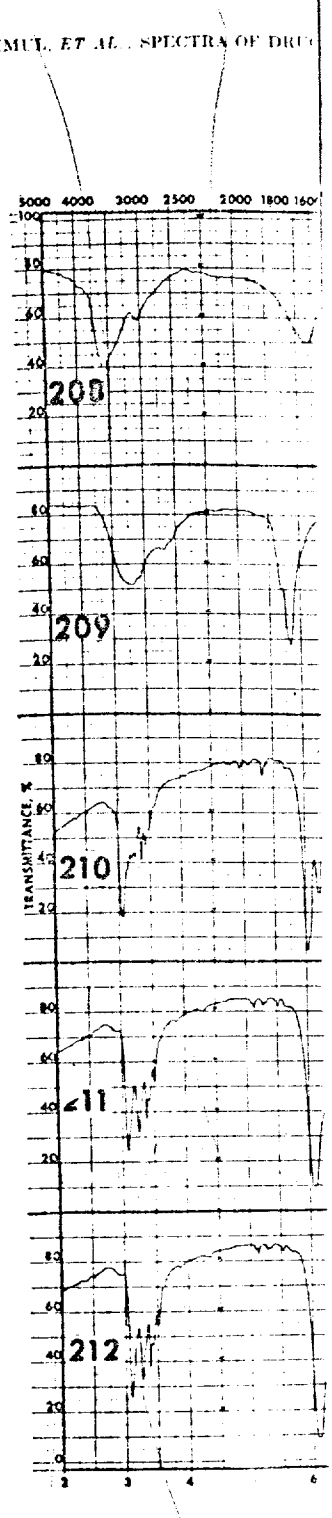
Table 1. (*Continued*)

Name	No.	Conditions	Retrieval No.	Page No.
Metformin Hydrochloride	439	isoC$_5$H$_{11}$-CH$_3$OH-isoC$_5$H$_{11}$ (0.6:0.4:5.0); KCl	4036	971
Methiramine	440	CS$_2$, C$_2$Cl$_4$ solutions	8211	971
Methylbarbital	441	C$_6$H$_6$-CH$_3$OH; KBr	8110	971
Metoxobarbital	442	CHCl$_3$; KBr	8173	971
Metrobarbital	443	C$_6$H$_6$-MeOH (1:1); KBr	8187	972
Mexobutiodil	444	CS$_2$, C$_2$Cl$_4$ solutions	3031	972
Nanobutiodil	445	Direct KBr	3035	972
Nentolamine Methanesulfonate	446	Mull	8190	972
Neosynephrine Hydrochloride	447	*n*C$_3$H$_7$OH-isoC$_5$H$_{11}$ (0.4:0.6); KCl	4035	972
Norephedrine Hydrochloride	448	EtOH-isoC$_5$H$_{11}$ (1:3); KCl	4033	973
Norepinephrine Hydrochloride	449	CH$_3$OH-C$_6$H$_6$-isoC$_5$H$_{11}$-C$_5$H$_{11}$ (0.2:0.2:0.6:5.0); KCl	4068	973
Norphenylramidol Hydrochloride	450	C$_6$H$_6$OH-isoC$_5$H$_{11}$ (1:3); KCl	8093	973
Phenylramidol Salicylate	451	Direct KBr	8115	973
Physostigmine	452	CHCl$_3$; KBr	7017	973
Picric Acid	453	EtOH; KBr	4065	974
Piperidolate	454	CHCl$_3$-isoC$_5$H$_{11}$-C$_5$H$_{11}$ (0.5:0.5:5.0); KBr	8098	974
Placidyl	455	Film	1016	974
Poldine Methosulfate	456	Direct KBr	8088	974
Polyethylene Glycol tert-Dodecylthioether	457	C$_2$Cl$_4$ solutions	2042	974
Polythiazide	458	Direct KBr	8197	975
Potassium Aluminum Sulfate	478	Direct KBr	0039	979
Potassium Aspartate	459	Direct KBr	2028	975
Potassium Bromate	469	Direct KBr	0015	975
Potassium Chlorate	461	Direct KBr	0027	975
Potassium Iodate	462	Direct KBr	0028	975
Potassium Perchlorate	463	Direct KBr	0018	976
Potassium Teriodate	464	Direct KBr	0019	976
Potassium Sulfate, Anhydrous	479	Direct KBr	0030	979
Potassium Warfarin	465	Direct KBr	8096	976
Potato Starch	466	Direct KBr	1015	976
Pregnenolone Acetate	467	EtOAc-isoC$_5$H$_{11}$-isoC$_5$H$_{11}$ (0.5:0.5:5.0); KBr	6043	976
Preludin	468	Direct KBr	8091	977
Probarbital	469	EtOH-H$_2$O; KBr	8185	977
Procaine Hydrochloride	470	Direct KCl	4051	977
Progesterone	471	CS$_2$, C$_2$Cl$_4$ solutions	6049	977
Prochlorperazine Maleate	472	EtOH-C$_6$H$_{14}$ (2:3); KBr	8206	977
1,2-Propanediol Acetate	473	Film	1001	978
Propanol	474	Vapor	1029	978
1-(4-hydroxyphenyl)-1-Propanone	475	*n*C$_3$H$_7$OH-C$_6$H$_{14}$-*n*C$_5$H$_{11}$ (0.2:0.8:4.0); KBr	3017	978
Propionyl Erythromycin Lauryl Sulfate	476	*n*C$_3$H$_7$-isoC$_5$H$_{11}$-isoC$_5$H$_{11}$ (0.2:0.8:3.0); KBr	2020	978
1-Propoxyphene-2-naphthalene Sulfonate Monohydrate	480	Direct KBr	4034	979
1-Propoxyphene-N-oxide-Hydrochloride	477	Direct KCl	4032	978
Prothipendyl Hydrochloride	481	*n*C$_3$H$_7$OH-isoC$_5$H$_{11}$-isoC$_5$H$_{11}$ (0.2:0.8:4.0); KCl	8095	979
Pyridine	482	Film	4099	979
Quinethazone	484	Direct KBr	8180	980
Quinidine	485	CHCl$_3$ solution	7013	980
Salicylic Acid	486	Direct KBr	3020	980
Sandoptal	487	EtOH-H$_2$O; KBr	8186	980

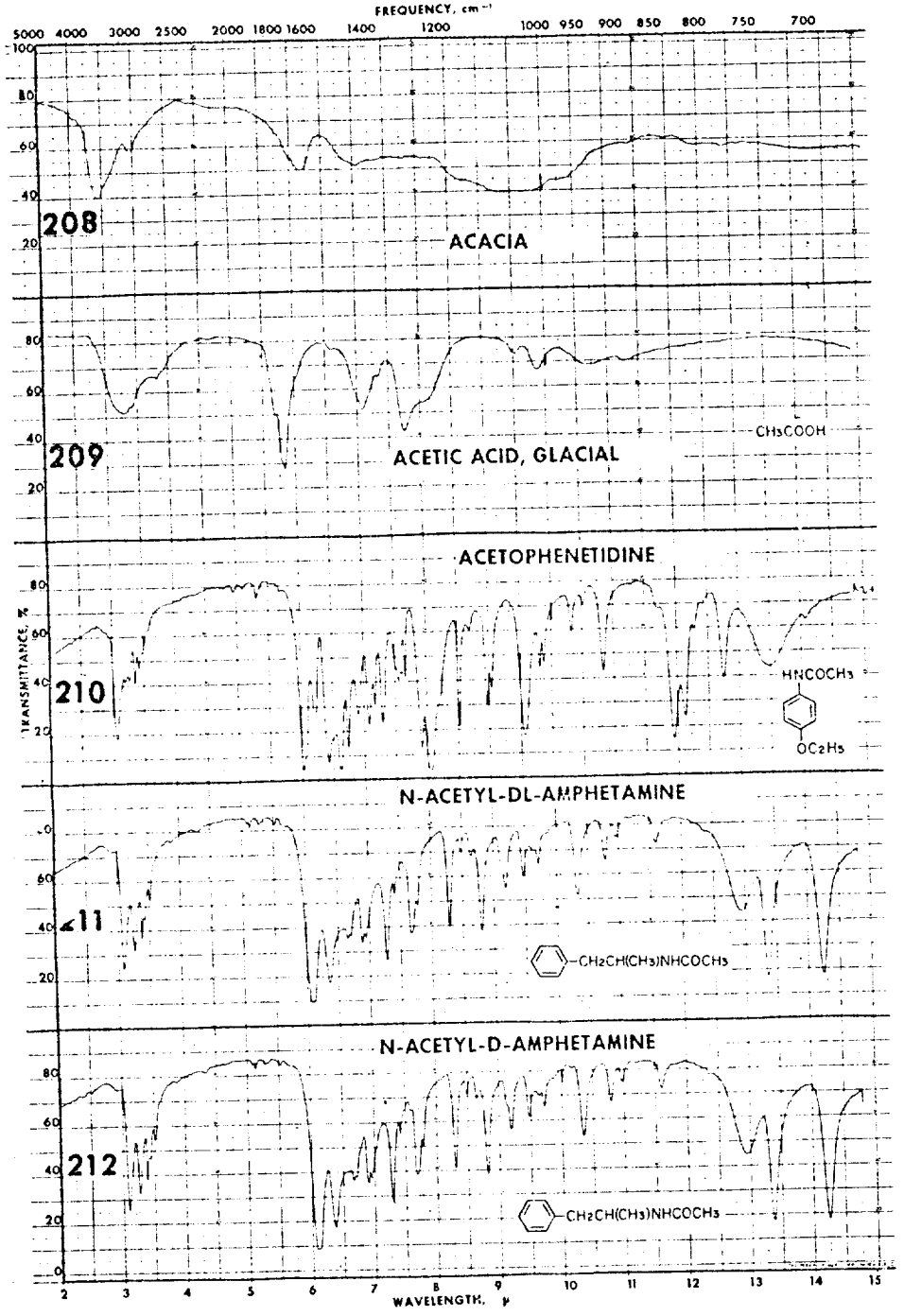
(*Continued*)

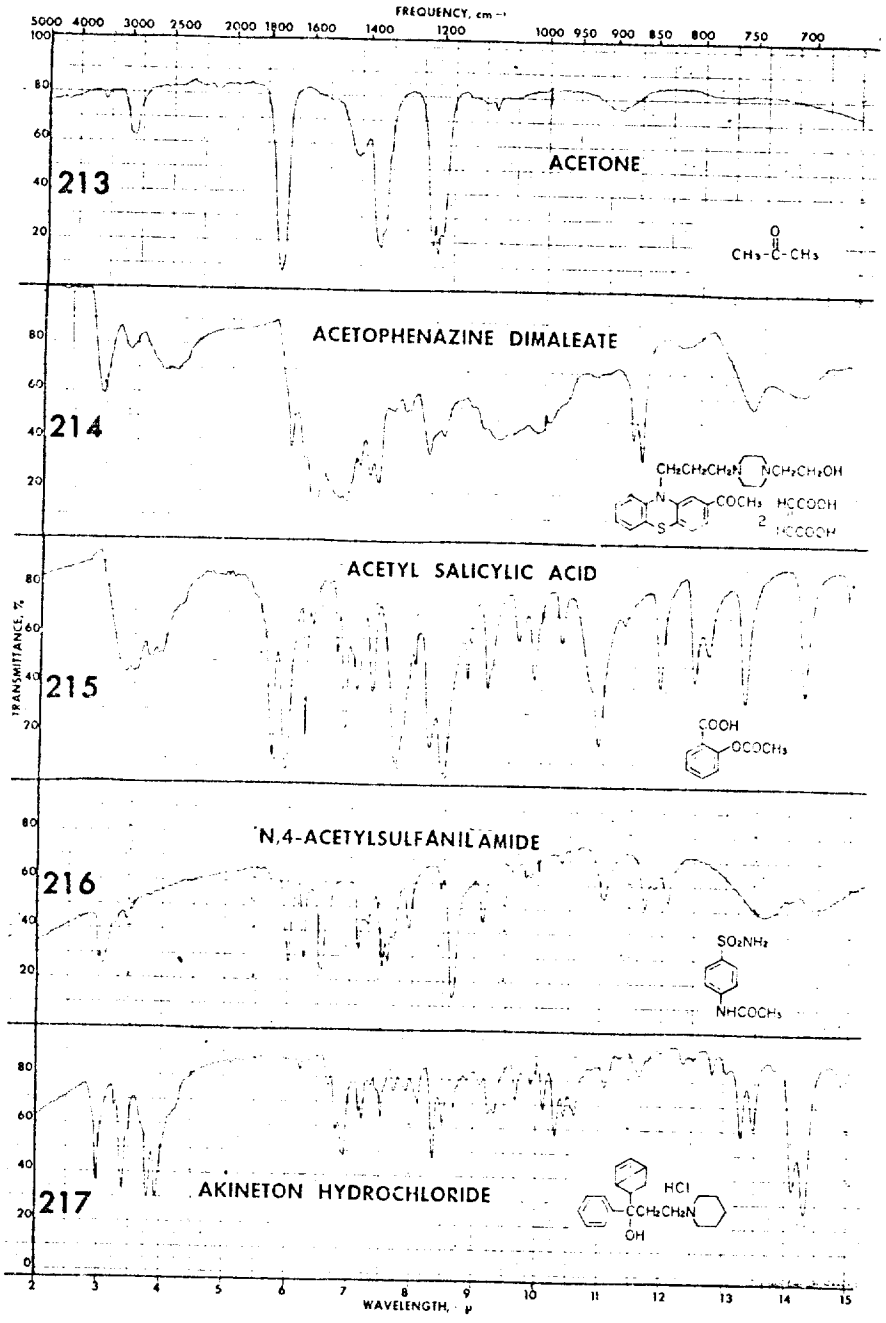
Table 1. *(Continued)*

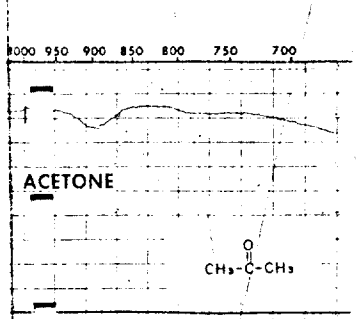
Name	No.	Conditions	Retrieval No.	Page No.
Scopolomine	488	CS_2 solution	7015	981
Secobarbital Sodium	489	Direct KBr	8150	981
Sitosterols	490	CS_2, C_2Cl_4 solutions	0036	981
beta-Sitosterol	491	CS_2, C_2Cl_4 solutions	6083	981
beta-Sitosterol	492	Et_2O; KBr	6084	981
Sodium Diacetate	503	Direct KBr	1019	984
Sodium Lauryl Sulfate	493	Direct KBr	2039	982
Sodium Nitrate	494	Direct KBr	0012	982
d-Sorbitol	495	EtOH; KBr	1017	982
Soya Sitosterols	496	CS_2, $CHCl_3$ solutions	0024	982
Soybean Oil	504	Film	0034	984
Sparteine Sulfate	497	Direct KBr	8127	982
Spironolactone	498	Me_2CO-isoC_5H_{12} (1:4); KBr	6050	983
Stanozolol	499	$CHCl_3$-*n*C_5H_{12} (1:3); KBr	6081	983
Starch	509	Direct KBr	1011	983
Stearic Acid	505	CS_2 solution	1043	984
Stearic Acid	506	CS_2; KBr	1046	984
Stearic Acid	501	Direct KBr	1007	983
Stigmasterol	502	CS_2, C_2Cl_4 solutions	6051	983
Succinyl Choline Picrate	507	H_2O; KBr	4098	984
Sucrose	508	Direct KBr	1012	985
Sulfachloropyridazine	509	Direct KBr	8122	985
Sulfamethazole	510	EtOH-isoC_5H_{12}-isoC_5H_{11} (1:4:5); KBr	8133	985
Sulfaphenazole	511	EtOH-isoC_5H_{12}-isoC_5H_{11} (2:8:5); KBr	8132	985
Sulfobromophthalein Sodium	512	Direct KBr	4053	985
Taractan	513	Direct KBr	8181	986
Tenuate	514	Direct KCl	4056	986
Testosterone	515	isoC_5H_{12}; KBr	6074	986
Testosterone	516	CS_2, C_2Cl_4 solutions	6080	986
Testosterone Propionate	517	CS_2, C_2Cl_4 solutions	6078	986
Tetrachloroethylene	518	Film	1018	987
Tetrachloroethylene	519	Liquid cell, 1 mm	1039	987
Tialdeimide	520	Direct KBr	8126	987
Theobromum	521	EtOH, KBr	7016	987
Thiethylperazine Dimaleate	522	Direct KBr	8191	987
Thioguanine	523	Direct KBr	8183	988
Trans-Stilbene	525	EtOH; KBr	3041	988
Thioridazine Hydrochloride	526	Direct KCl	8161	988
Thonzonium Bromide	524	Direct KBr	8134	988
Thymol	527	Sublimed (vacuum); KBr	3029	988
Tofranil	528	EtOH-$CHCl_3$ (1:3); KBr	8121	989
O,O'-Di-Trifluoroacetyl-l-epinephrine	529	Me_2CO-Et_2O; KBr	4058	989
O,O'-Di-Trifluoroacetyl-l-norepinephrine	530	Me_2CO-CH_2Me_2; KBr	7023	989
Triboron Chloride	531	Direct KCl	2041	989
Trichlorocarbanilide	532	MeOH-CH_2-isoC_5H_{11} (0.5:0.5:5.0); KBr	1079	989
Trichlormethiazide	533	EtOH-isoC_5H_{12}-isoC_5H_{11} (0.2:0.8:5.0); KBr	8160	990
Triethylamine	538	CS_2 solution (redistilled)	2053	991
Trifluoromazine	534	CS_2, C_2Cl_4 solutions	8141	990
Trifluoromazine Hydrochloride	535	Direct KCl	8142	990
3,5,3'-Triiodothyropropionic Acid	536	Direct KBr	4054	990
Trimeprazine	537	iso$C_5H_{11}OH$; KBr	4088	990
Uracil Mustard	539	Direct KBr	8166	991
Urea	540	Direct KBr	2052	991
Vanillic Acid Diethylamide	541	Direct KBr	4055	991
Xanthiol Hydrochloride	542	Direct KCl	8207	991

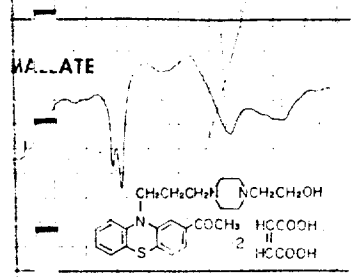


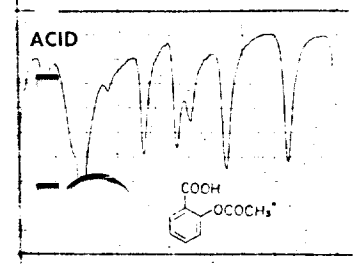
ditions	Retrieval No.	Page No.
olution	7015	981
KBr	8150	981
l_4 solutions	0036	981
l_4 solutions	6083	981
Br	6084	981
KBr.	1019	981
KBr	2039	982
Br	0012	982
KBr	1017	982
Cl_3 solutions	0024	982
	0034	981
KBr	8127	982
C_6H_{14} (1:4); KBr	6050	983
C_7H_{16} (1:3); KBr	6081	983
Br	1011	983
olution	1043	984
KBr	1046	984
Br	1007	983
l_4 solutions	6051	983
Br	1098	984
KBr	1012	985
KBr	8122	985
C_6H_{14}-isoC_8H_{18} (4); KBr	8133	985
C_6H_{14}-isoC_8H_{18} (8:5); KBr	8132	985
KBr	4053	985
Br	8181	986
KCl	4056	986
H_{14}; KBr	6074	986
CCl_4 solutions	6080	986
tions	6078	986
	1018	987
mm	1039	987
KBr	8126	987
KBr	7016	987
Br	8191	987
Br	8183	988
H_4, KBr	3041	988
KCl	8161	988
KBr	8134	988
d (vacuum); KBr	3029	988
C_7H_{16} (1:3); KBr	8121	989
CO-Et_2O; KBr	4058	989
CO-C_4H_9Me; KBr	7023	989
KCl	2044	989
C_6H_{14}-isoC_8H_{18} (7 .5:5.0); KBr	4079	989
H_{14}-isoC_8H_{18}-isoC_8H_{18} (2:0.8:5.0); KBr	8160	990
solution (redistilled)	2053	991
l_4 solutions	8141	990
KCl	8142	990
KBr	4054	990
H_5OH; KBr	4088	990
KBr	8166	991
KBr	2052	991
KBr	4055	991
KCl	8207	991

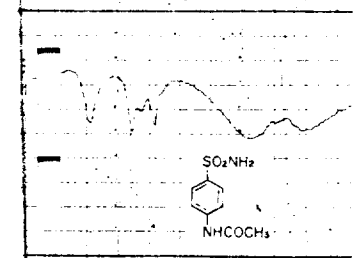


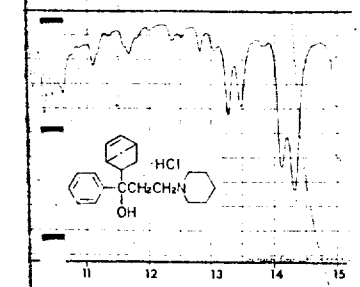

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
213
ACETONE
CH₃-C-CH₃
214
ACETOPHENAZINE DIMALEATE
215
ACETYL SALICYLIC ACID
COOH
OCOCH₃
TRANSMITTANCE, %
216
N,4-ACETYLSULFANILAMIDE
SO₂NH₂
NHCOCH₃
217
AKINETON HYDROCHLORIDE
HCl
OH
WAVELENGTH, μ

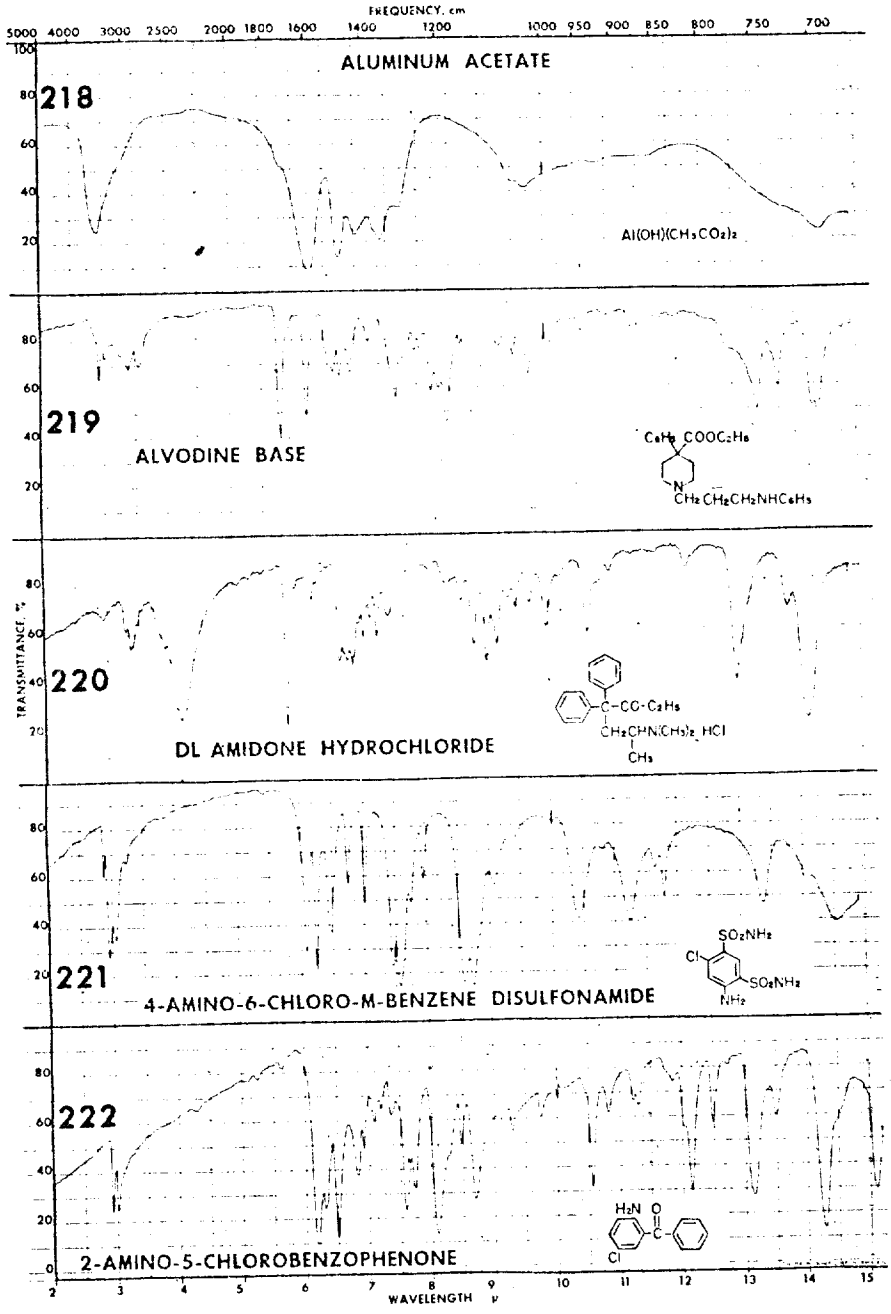

ACETONE
O
CH3-C-CH3

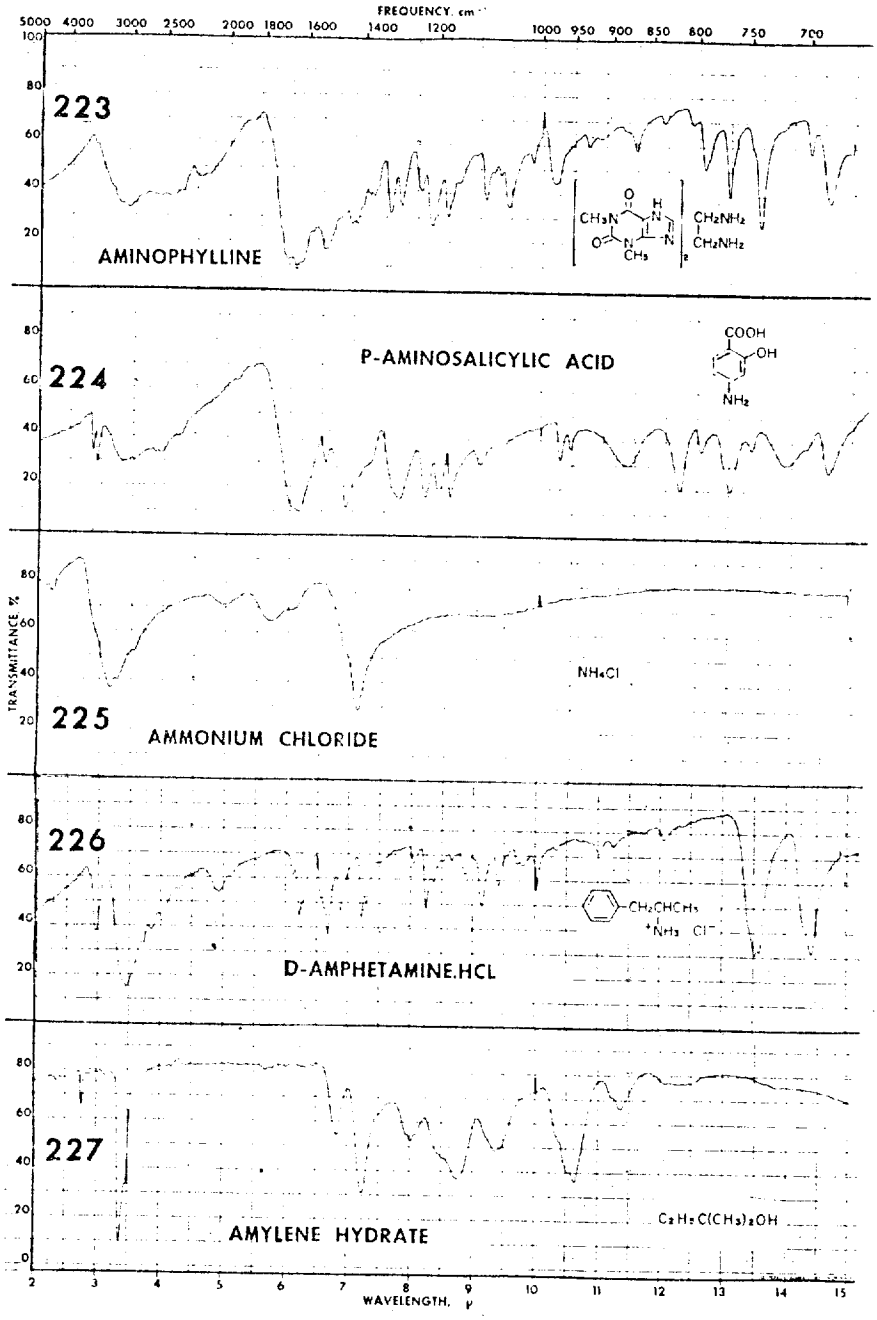

MA..ATE
CH3CH2CH2N
NCH2CH2OH
COCH3
2
HCCOOH
HCCOOH

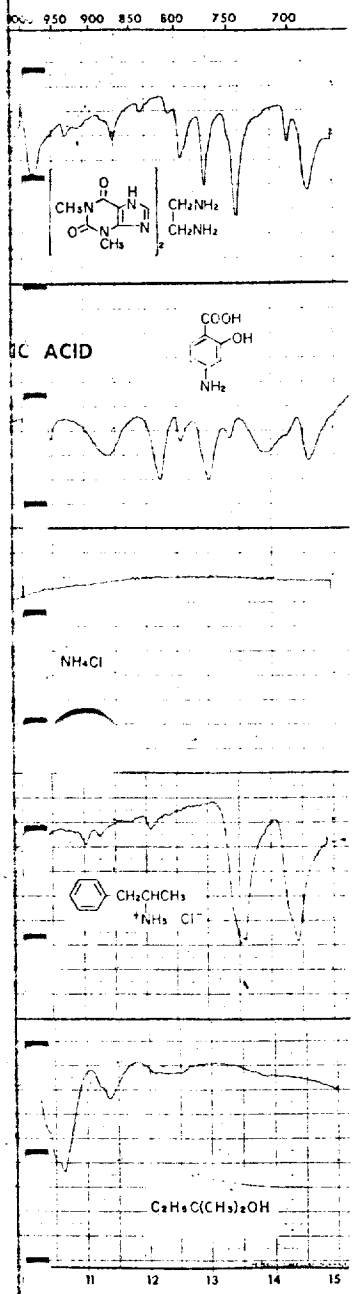

ACID
COOH
OCOCH3

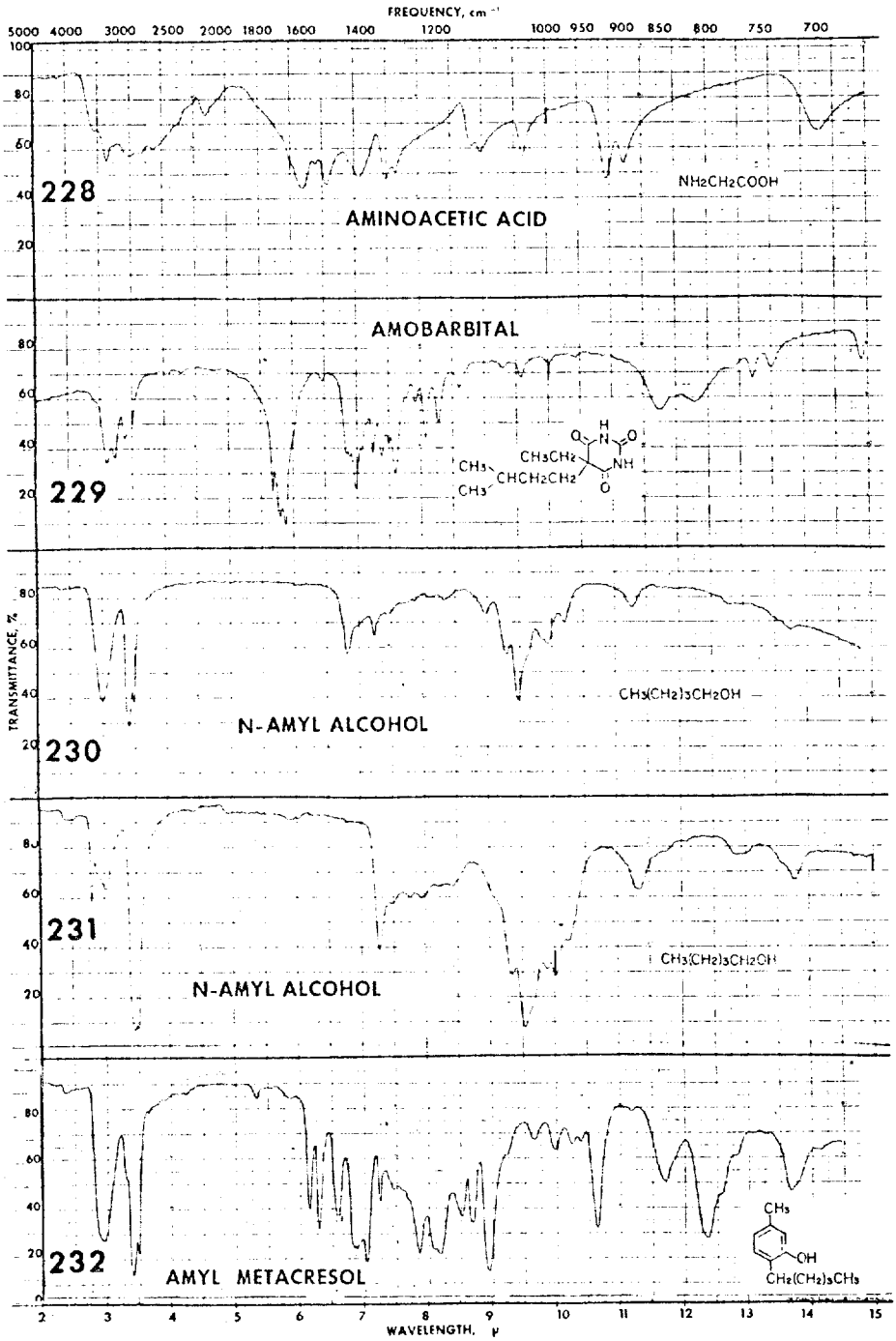

SO2NH2
NHCOCH3

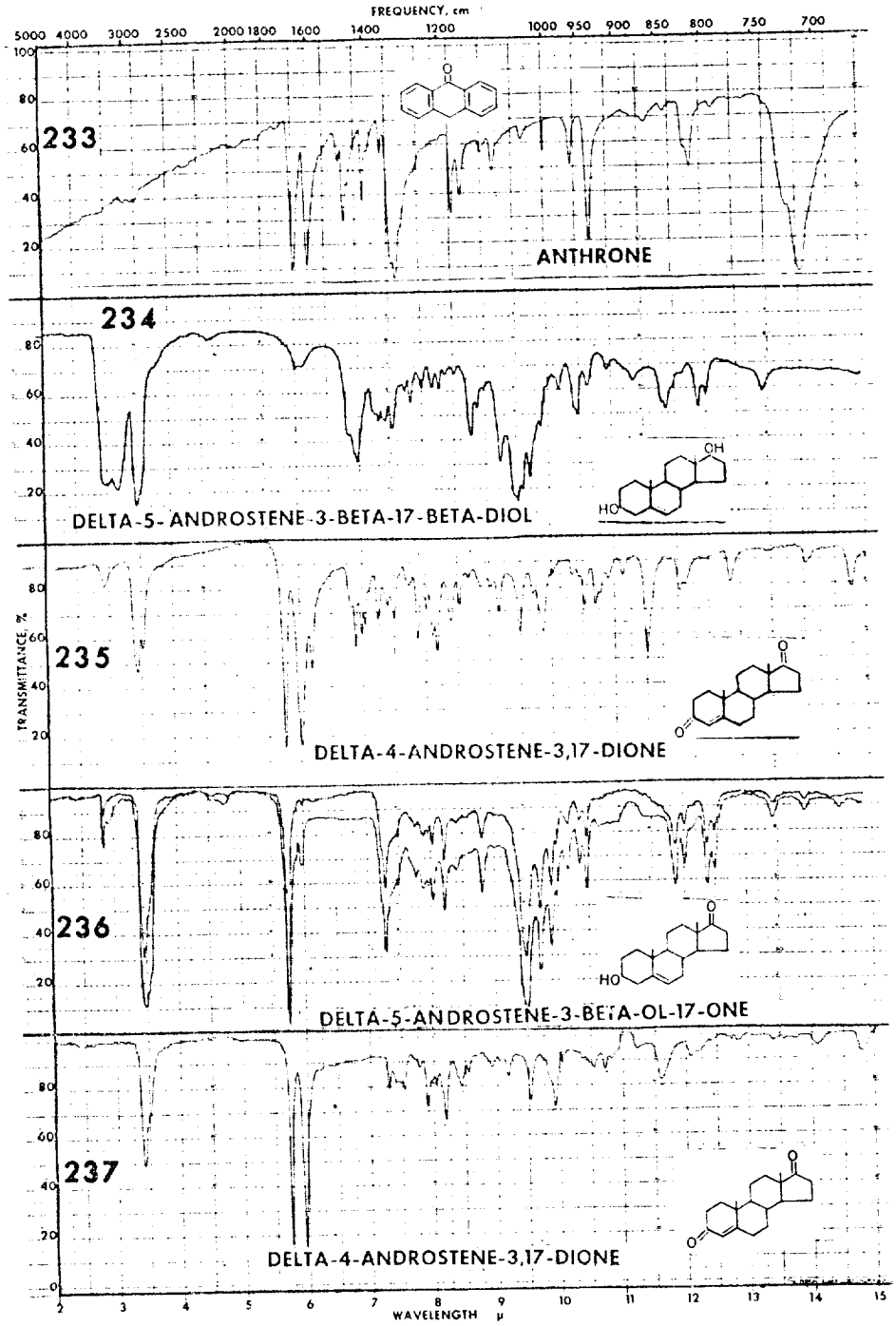

HCl
CCH2CH2N
OH
11
12
13
14
15

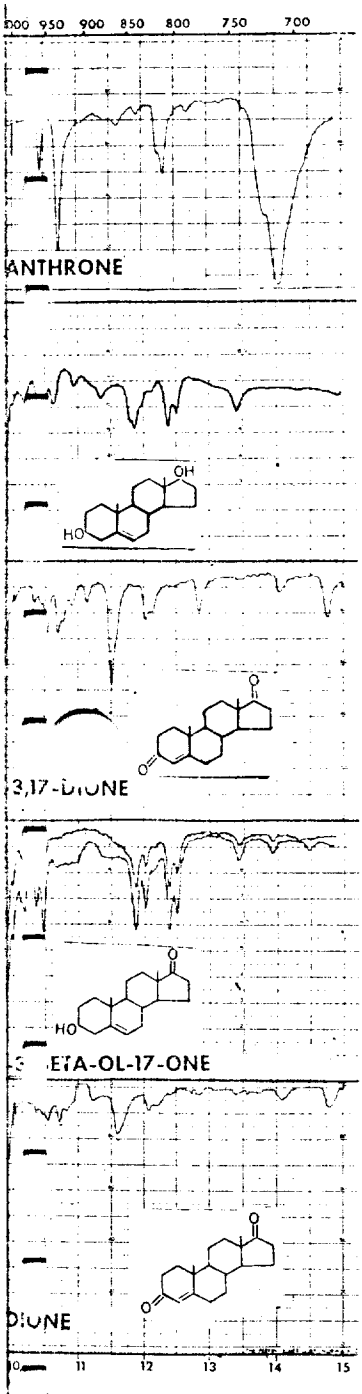

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
ALUMINUM ACETATE
218
Al(OH)(CH3CO2)2
219
ALVODINE BASE
C6H5 COOC2H5
N
CH2CH2CH2NHC6H5
220
DL AMIDONE HYDROCHLORIDE
C-CO-C2H5
CH2CHN(CH3)2 HCl
CH3
221
4-AMINO-6-CHLORO-M-BENZENE DISULFONAMIDE
SO2NH2
Cl
SO2NH2
NH2
222
2-AMINO-5-CHLOROBENZOPHENONE
H2N O
C
Cl
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH μ

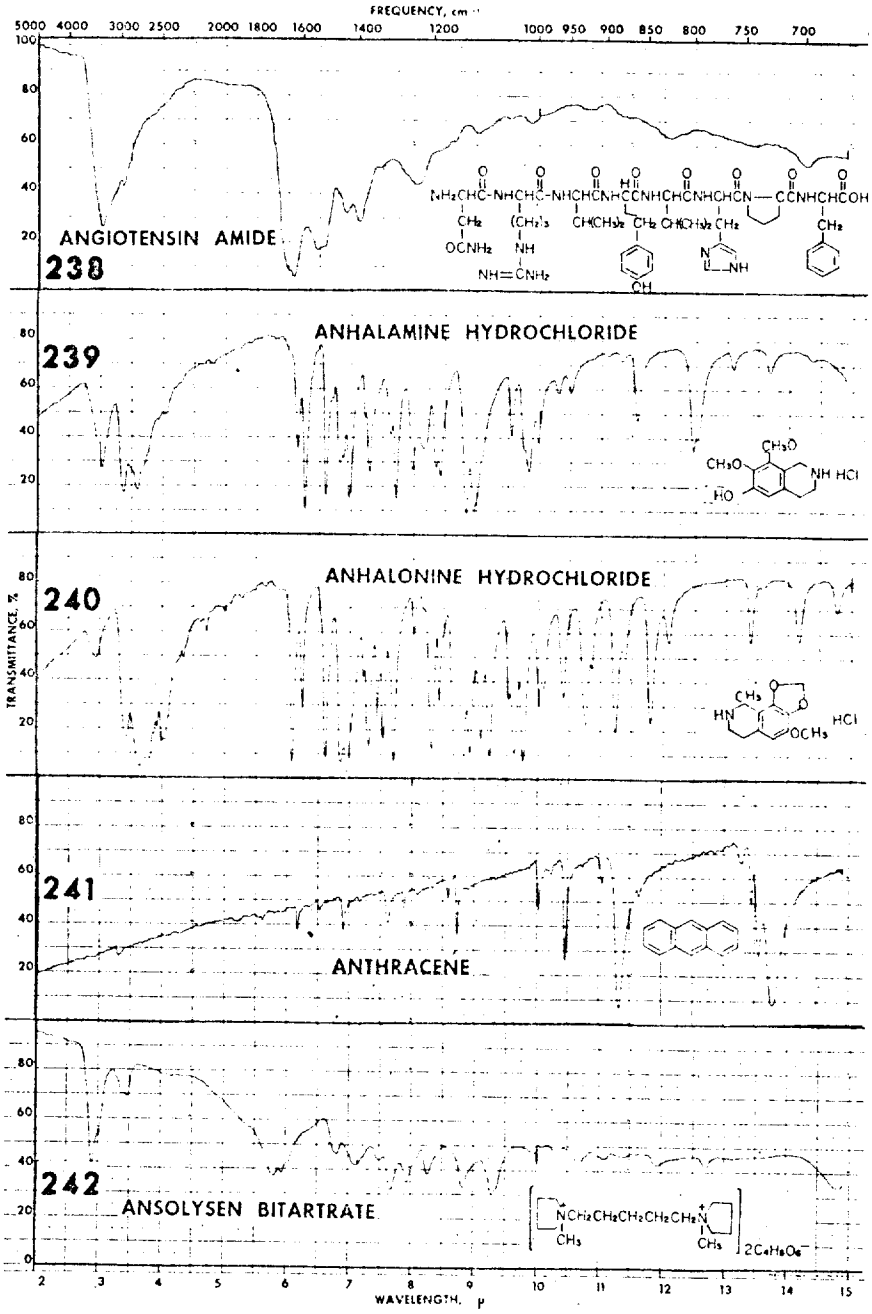

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
223
AMINOPHYLLINE
224
P-AMINOSALICYLIC ACID
225
AMMONIUM CHLORIDE
NH4Cl
226
D-AMPHETAMINE.HCL
227
AMYLENE HYDRATE
C2H5C(CH3)2OH
TRANSMITTANCE %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

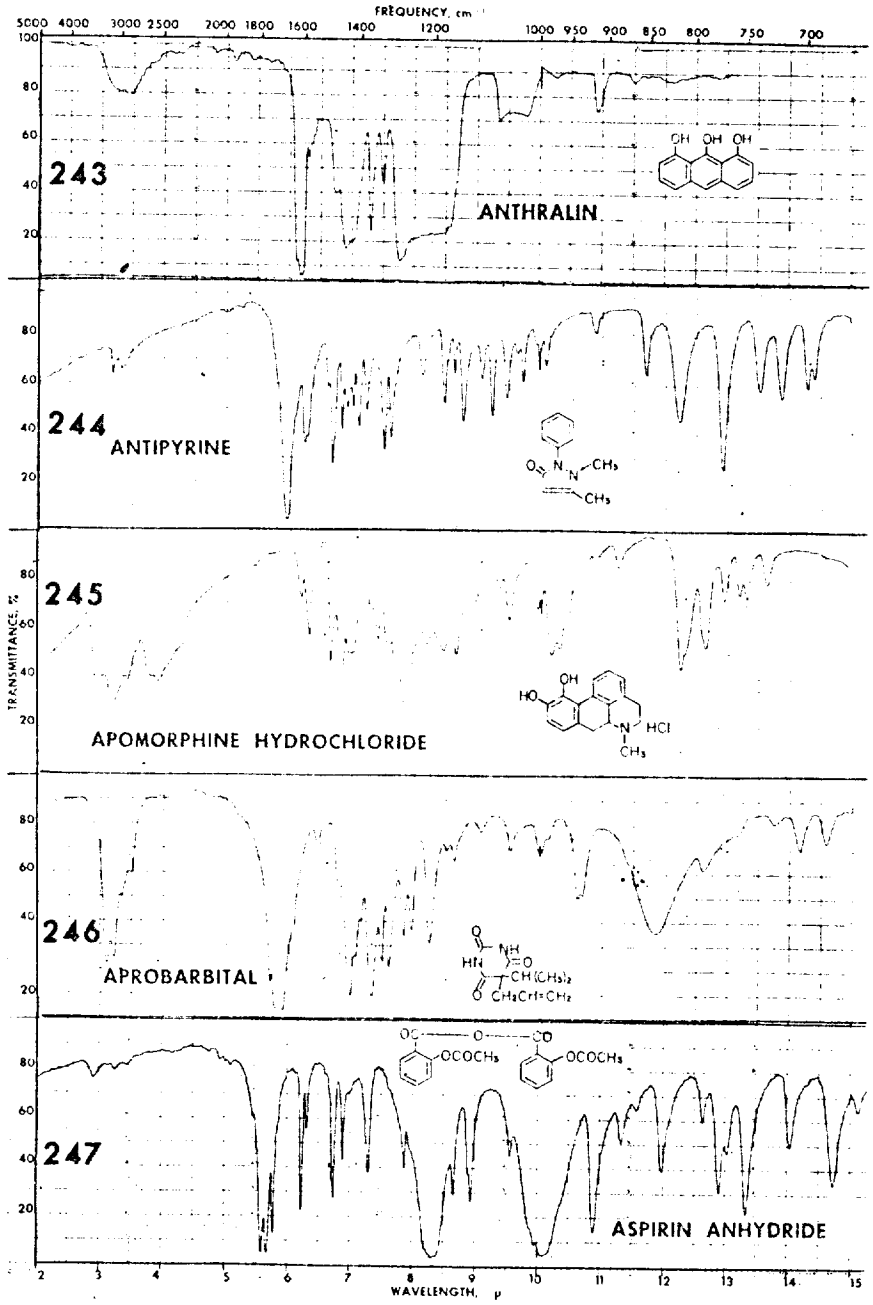

950 900 850 800 750 700
C ACID
NH4Cl
CH2CHCH3
+NH3 Cl−
C2H5C(CH3)2OH
11 12 13 14 15

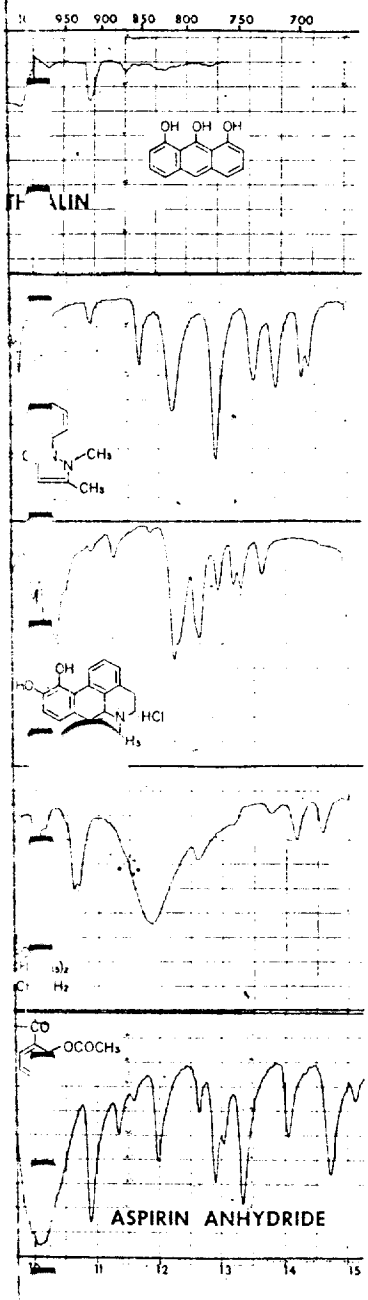

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
228
AMINOACETIC ACID
NH2CH2COOH
AMOBARBITAL
229
CH3CH2
CH3
CH3
CHCH2CH2
NH
TRANSMITTANCE, %
230
N-AMYL ALCOHOL
CH3(CH2)3CH2OH
231
N-AMYL ALCOHOL
CH3(CH2)3CH2OH
232
AMYL METACRESOL
CH3
OH
CH2(CH2)3CH3
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

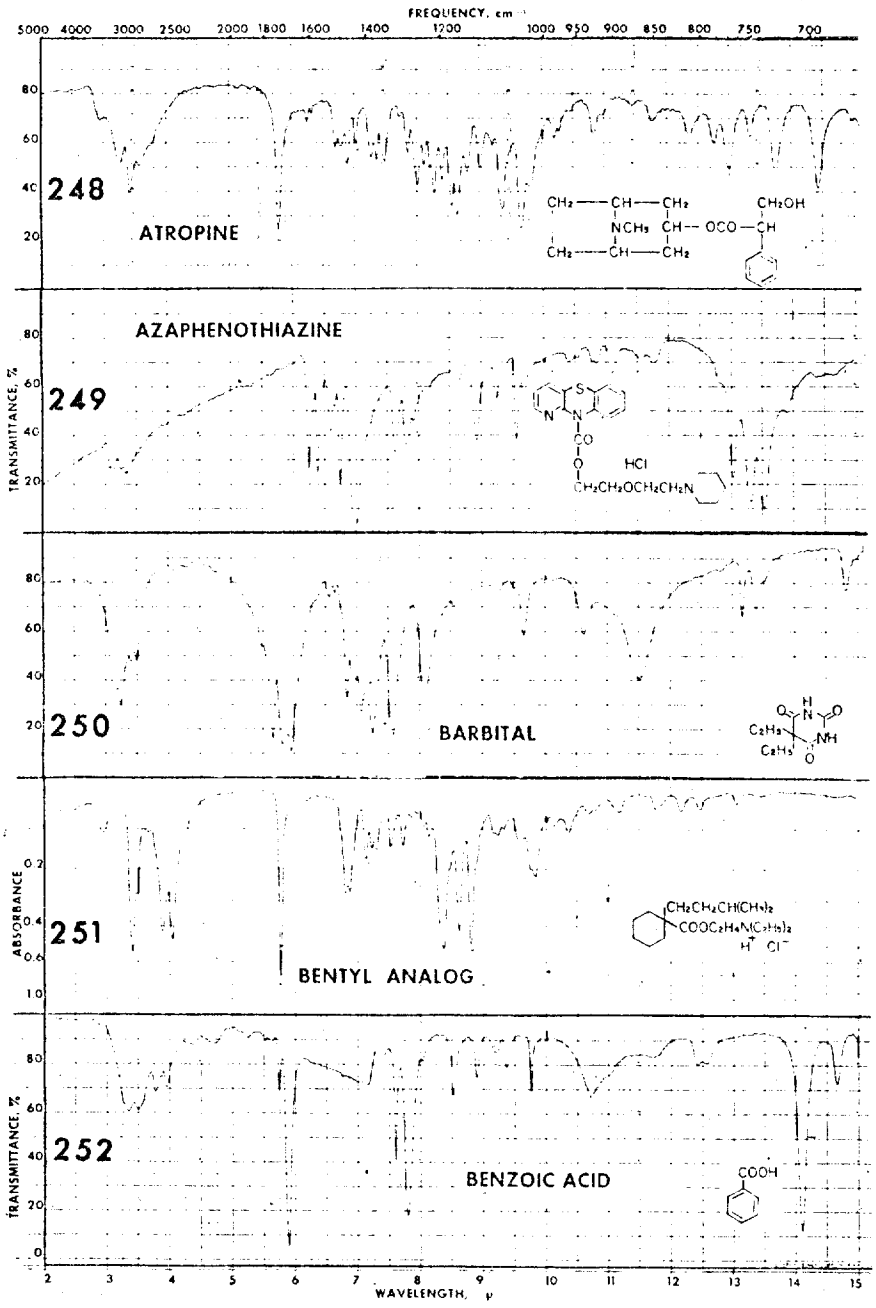

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
233
ANTHRONE
234
DELTA-5-ANDROSTENE-3-BETA-17-BETA-DIOL
OH
HO
TRANSMITTANCE, %
235
DELTA-4-ANDROSTENE-3,17-DIONE
236
DELTA-5-ANDROSTENE-3-BETA-OL-17-ONE
237
DELTA-4-ANDROSTENE-3,17-DIONE
WAVELENGTH μ

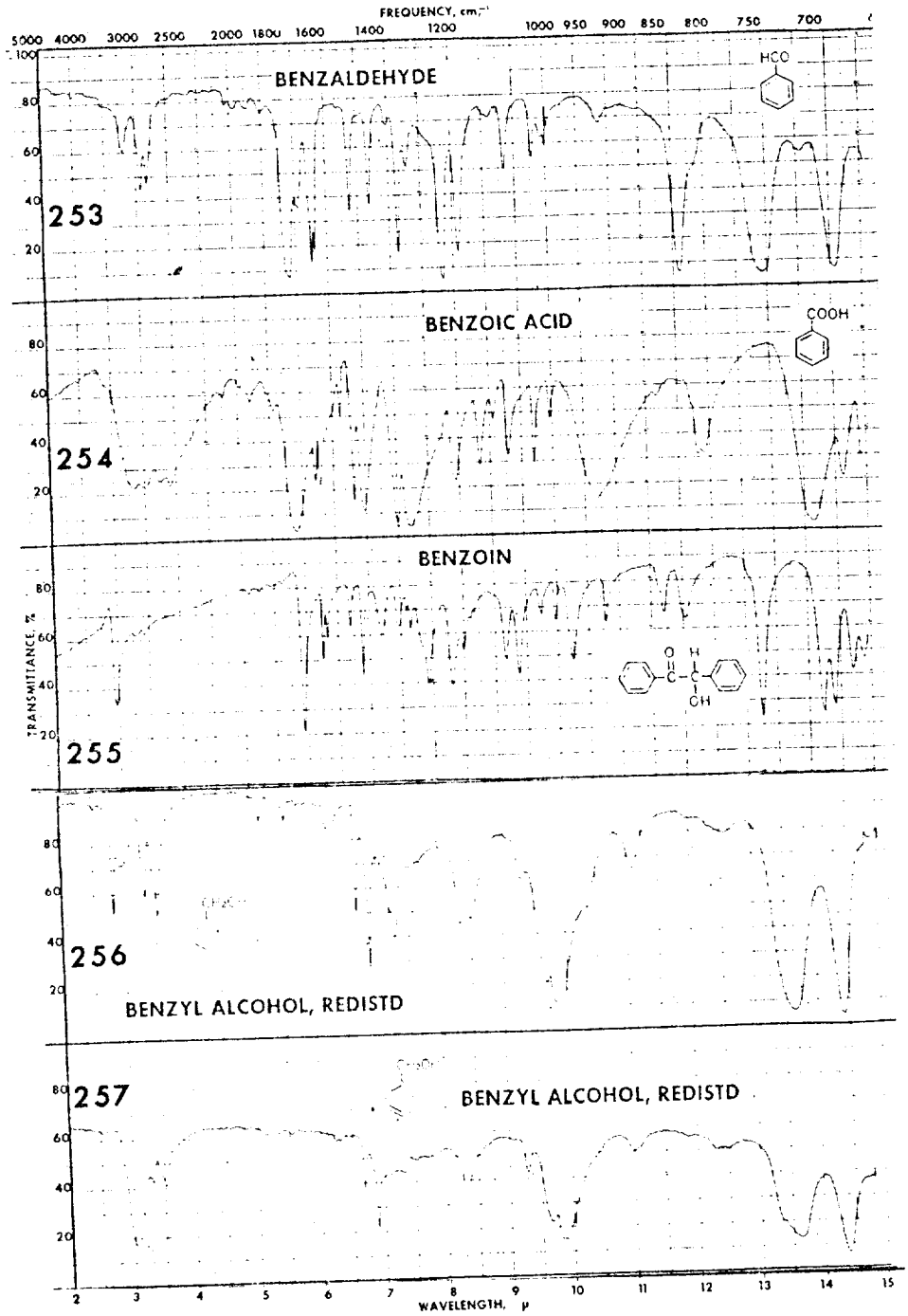

950 900 850 800 750 700
ANTHRONE
OH
HO
3,17-DIONE
HO
ETA-OL-17-ONE
DIONE
10 11 12 13 14 15

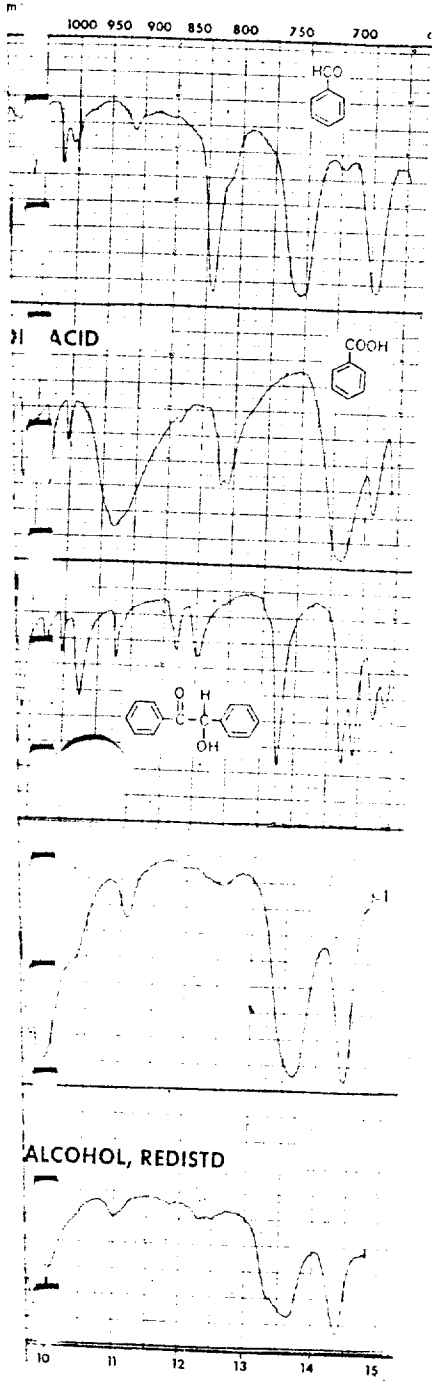

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ANGIOTENSIN AMIDE
238
ANHALAMINE HYDROCHLORIDE
239
ANHALONINE HYDROCHLORIDE
240
TRANSMITTANCE, %
ANTHRACENE
241
ANSOLYSEN BITARTRATE
242
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

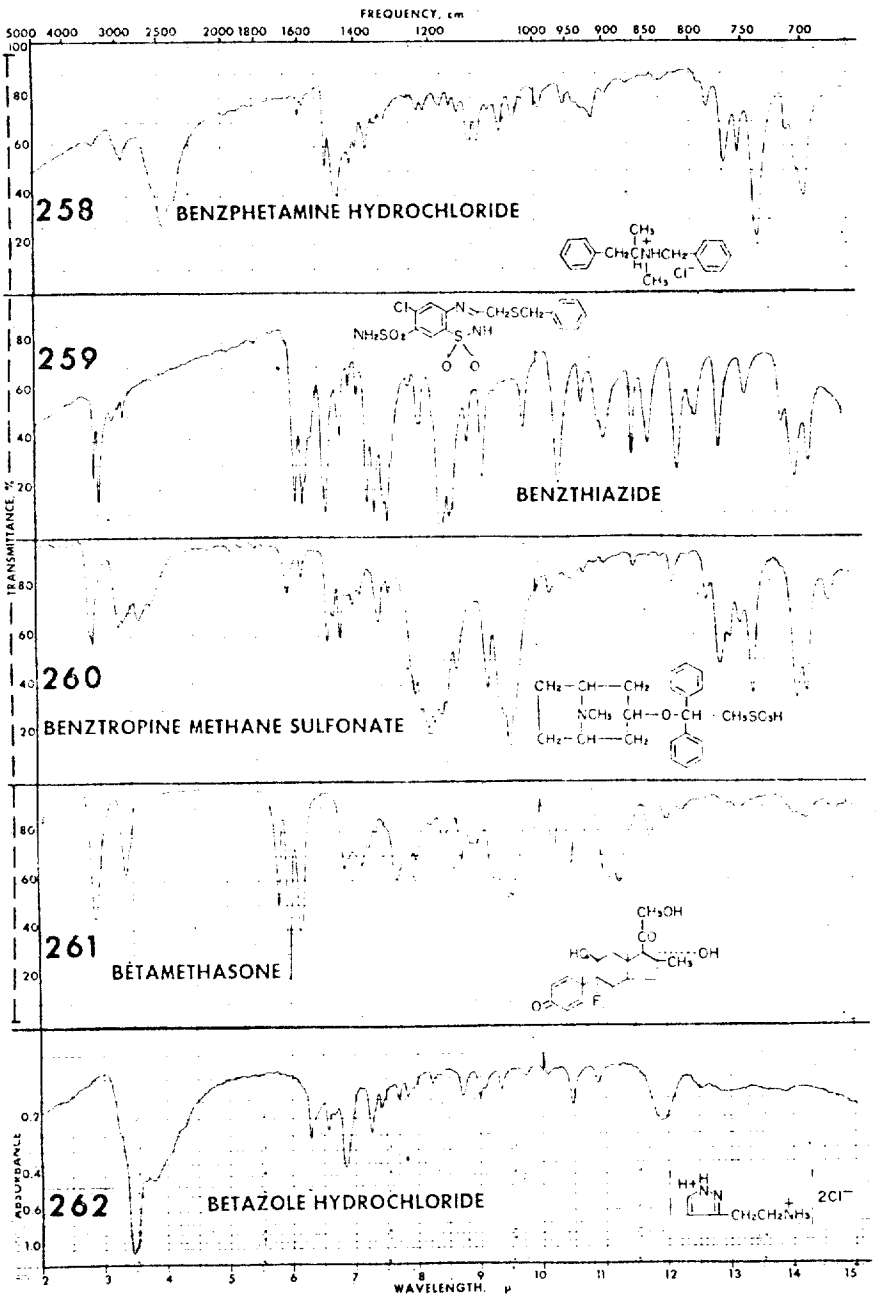

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
243
ANTHRALIN
OH OH OH
244
ANTIPYRINE
245
APOMORPHINE HYDROCHLORIDE
TRANSMITTANCE, %
246
APROBARBITAL
247
ASPIRIN ANHYDRIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

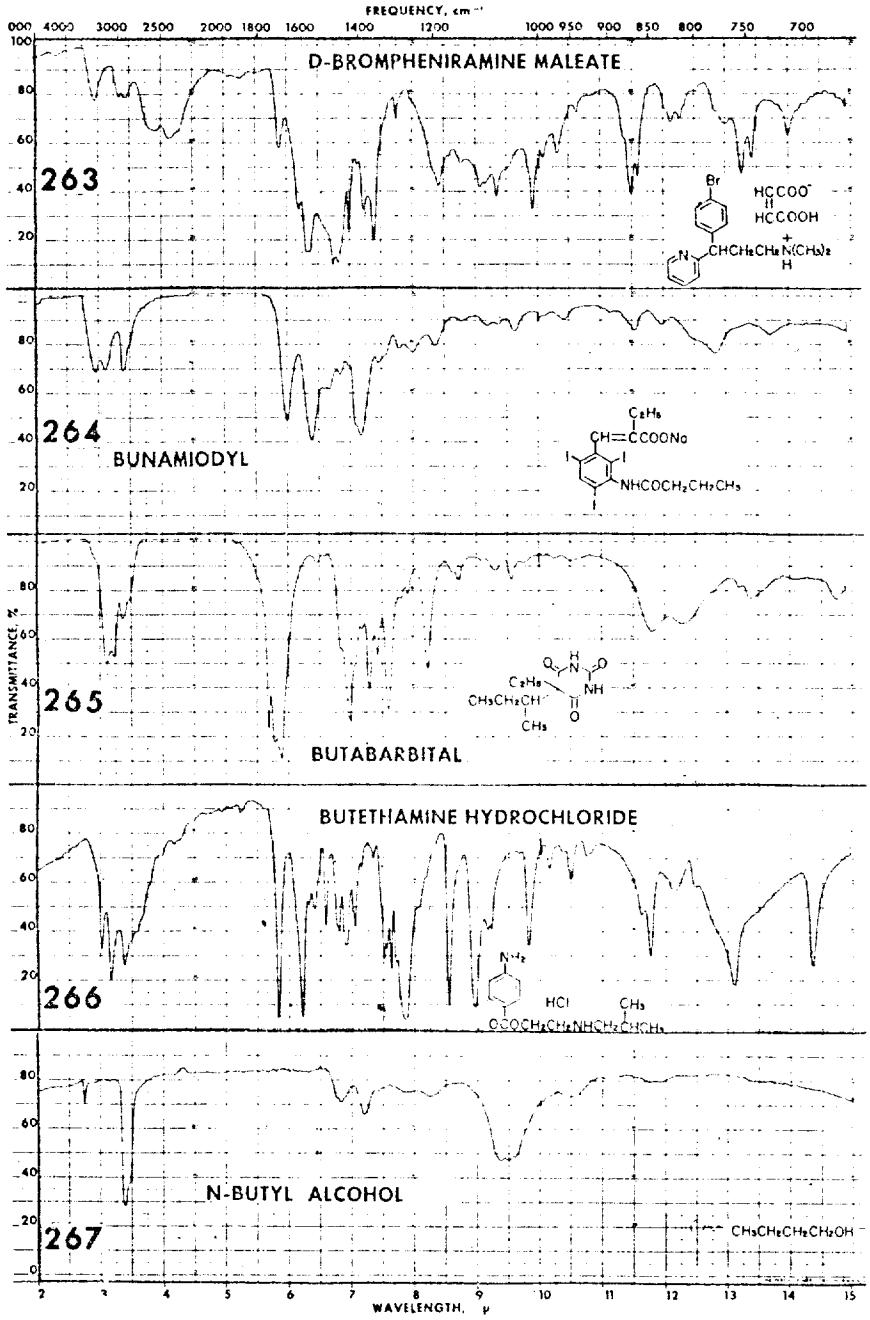

950 900 850 800 750 700
OH OH OH
ALIN
CH3
CH3
OH
HCl
H3
OCOCH3
ASPIRIN ANHYDRIDE
11 12 13 14 15

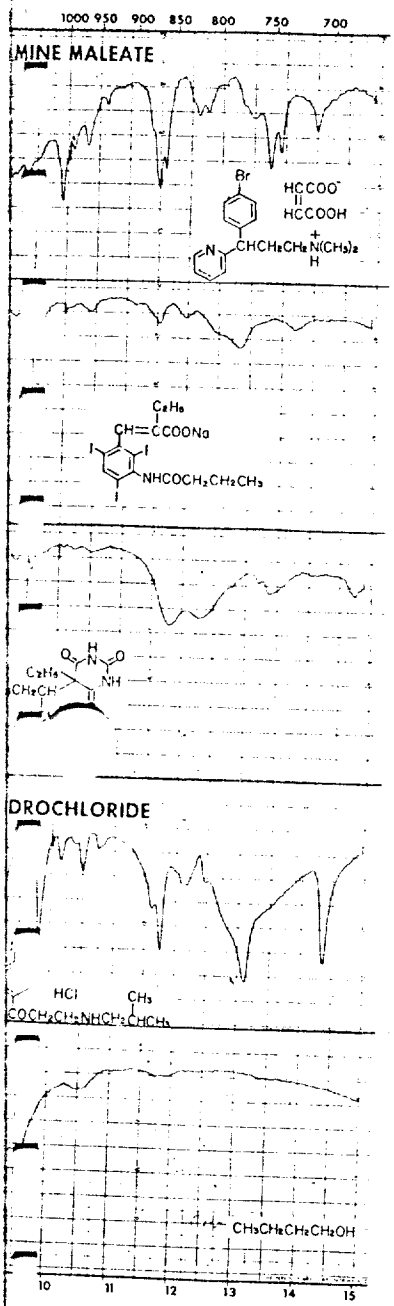

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
248
ATROPINE
CH2OH
NCH3
249
AZAPHENOTHIAZINE
TRANSMITTANCE, %
HCl
250
BARBITAL
251
BENTYL ANALOG
ABSORBANCE
252
BENZOIC ACID
COOH
TRANSMITTANCE, %
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

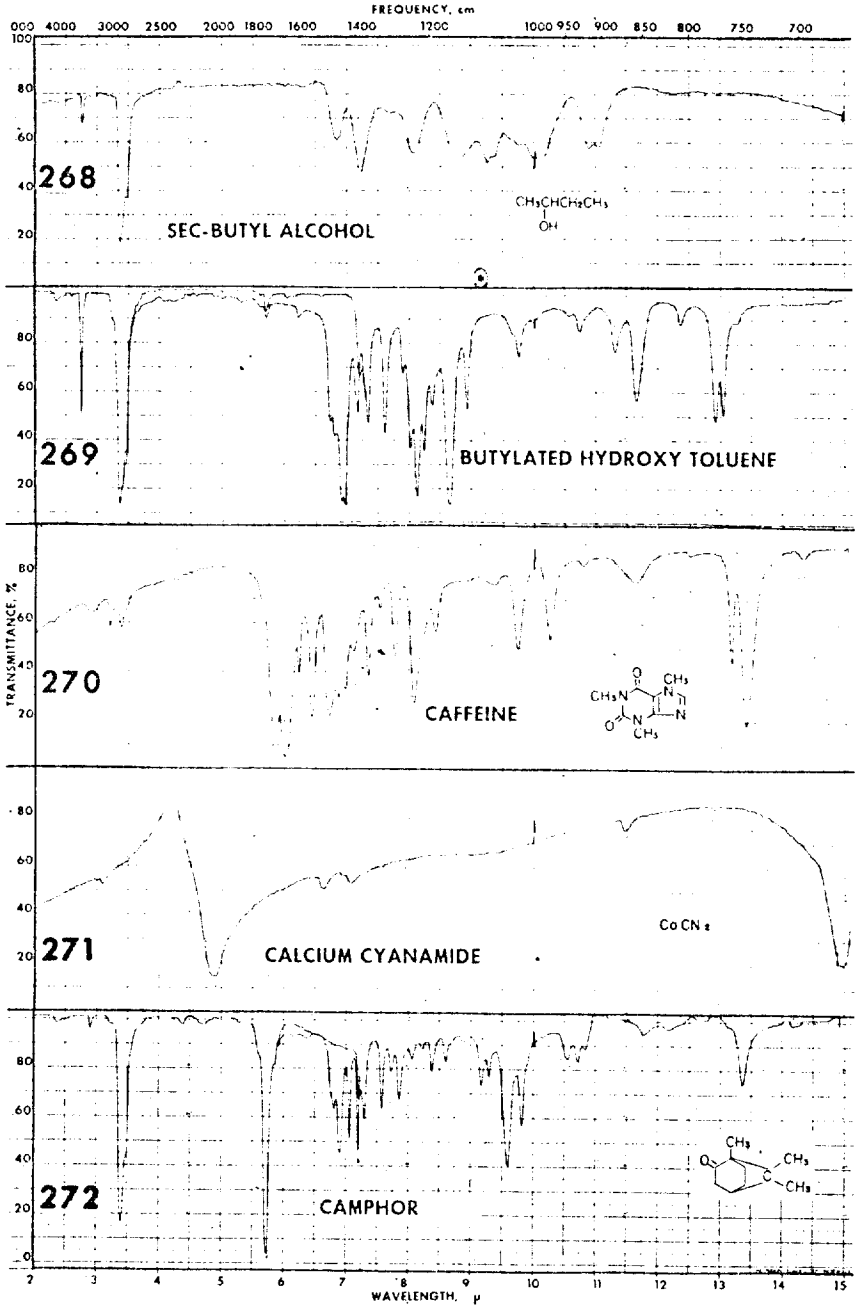

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
BENZALDEHYDE
HCO
253
BENZOIC ACID
COOH
254
BENZOIN
TRANSMITTANCE, %
255
256
BENZYL ALCOHOL, REDISTD
257
BENZYL ALCOHOL, REDISTD
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

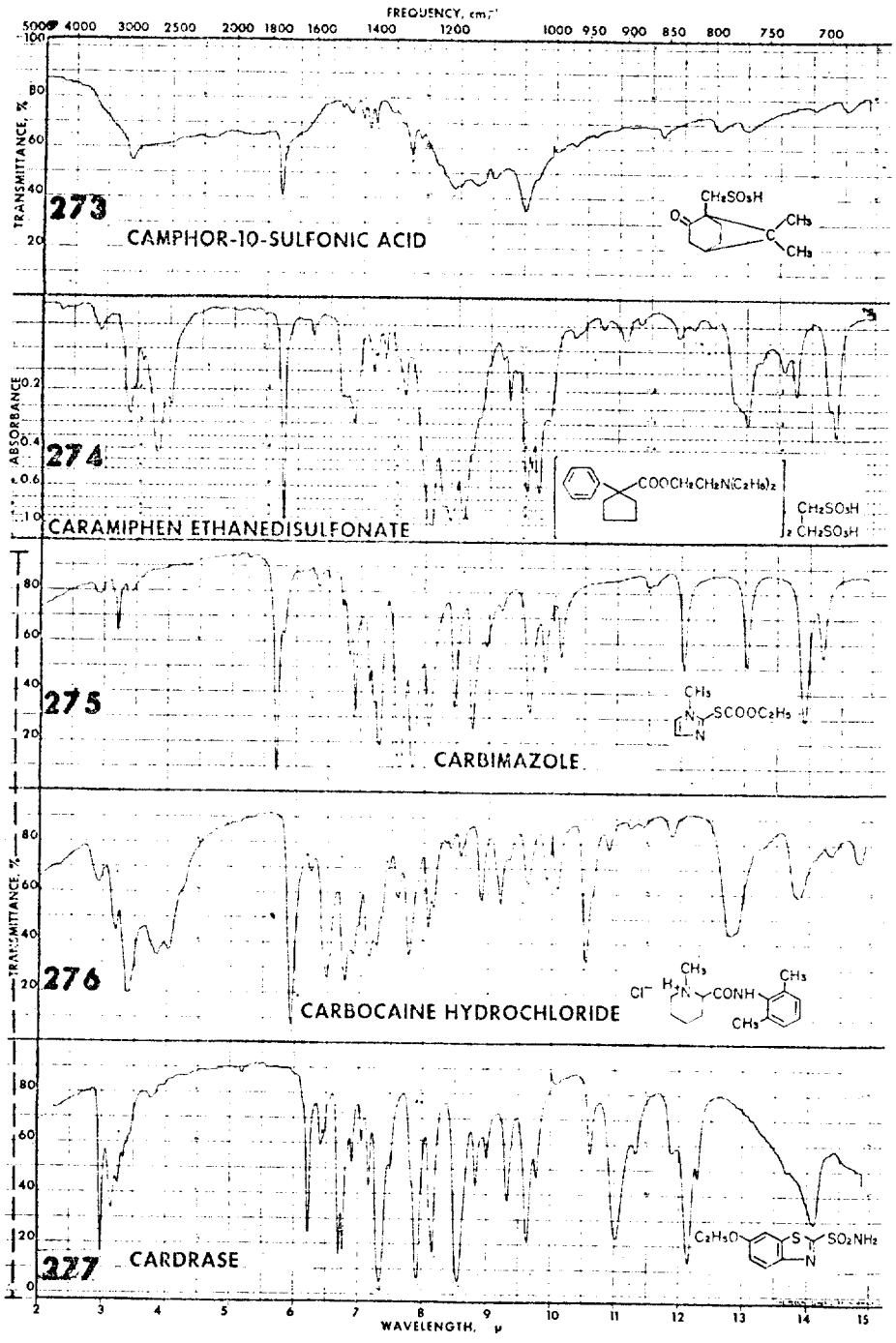

1000 950 900 850 800 750 700
HCO
ACID
COOH
ALCOHOL, REDISTD
10 11 12 13 14 15

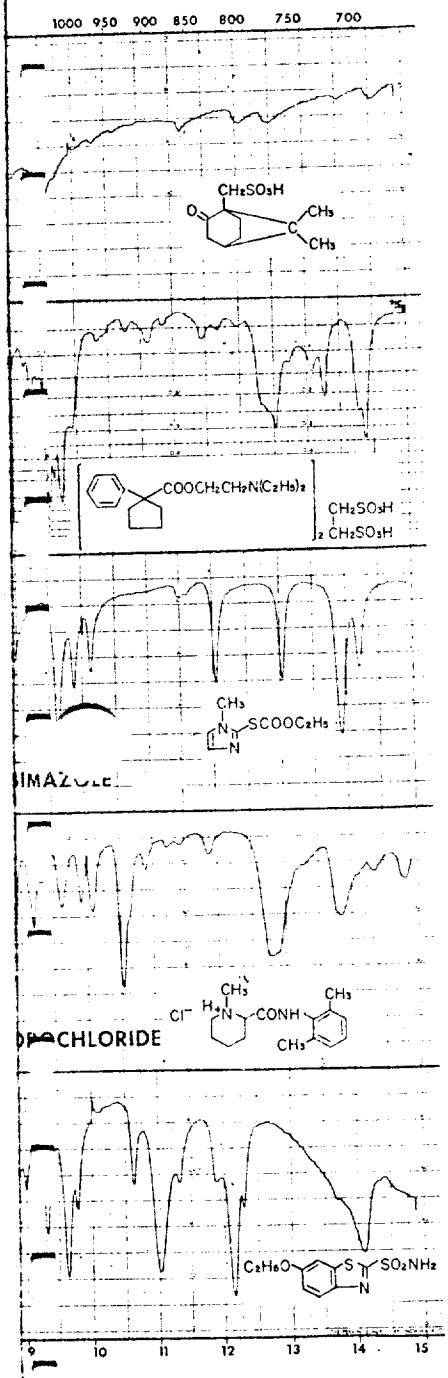

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
258
BENZPHETAMINE HYDROCHLORIDE
259
BENZTHIAZIDE
260
BENZTROPINE METHANE SULFONATE
261
BETAMETHASONE
262
BETAZOLE HYDROCHLORIDE
ABSORBANCE
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

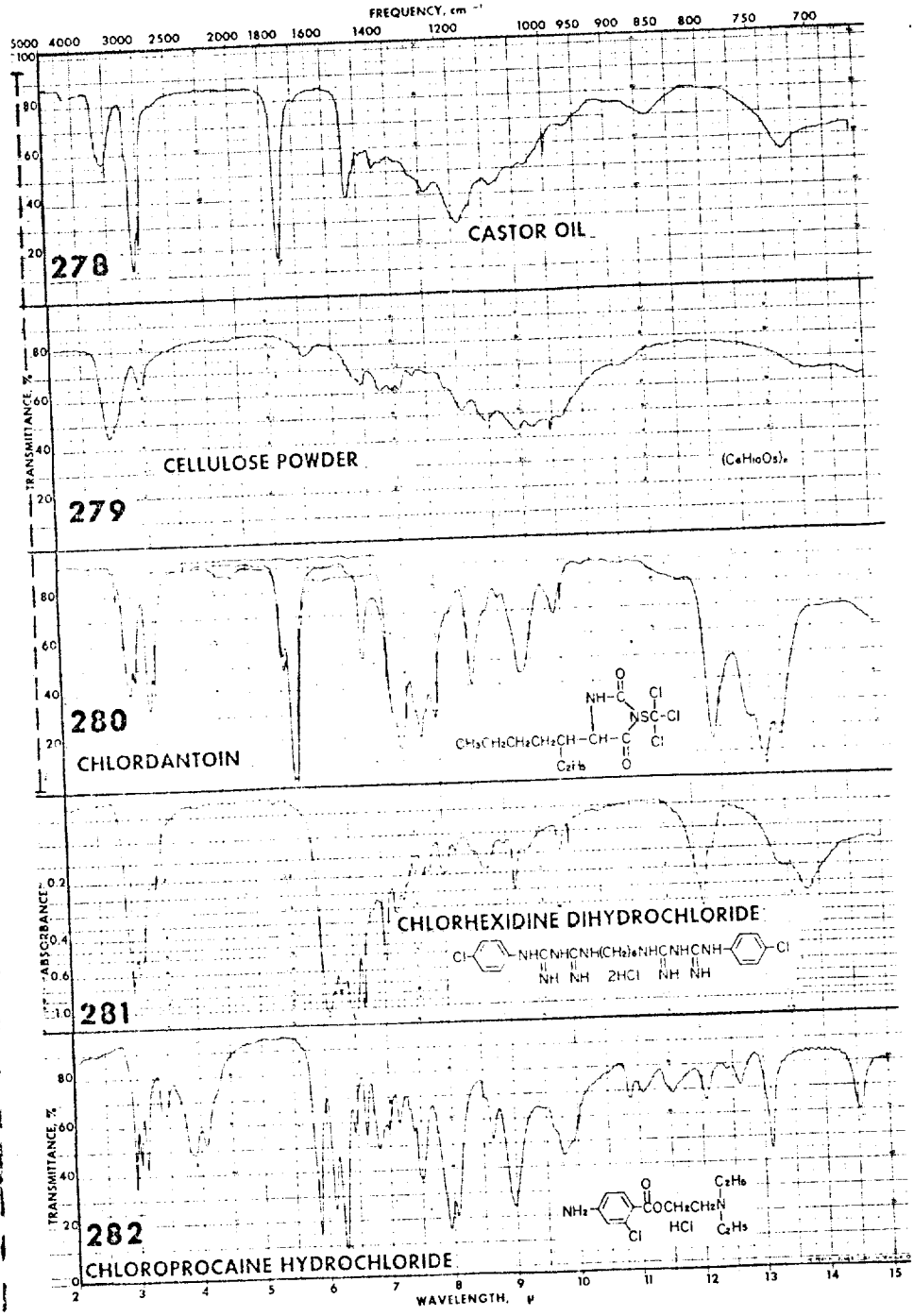

FREQUENCY, cm⁻¹
4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
D-BROMPHENIRAMINE MALEATE
263
HCCOO⁻
HCCOOH
BUNAMIODYL
264
BUTABARBITAL
265
BUTETHAMINE HYDROCHLORIDE
266
N-BUTYL ALCOHOL
267
CH₃CH₂CH₂CH₂OH
TRANSMITTANCE, %
WAVELENGTH, μ

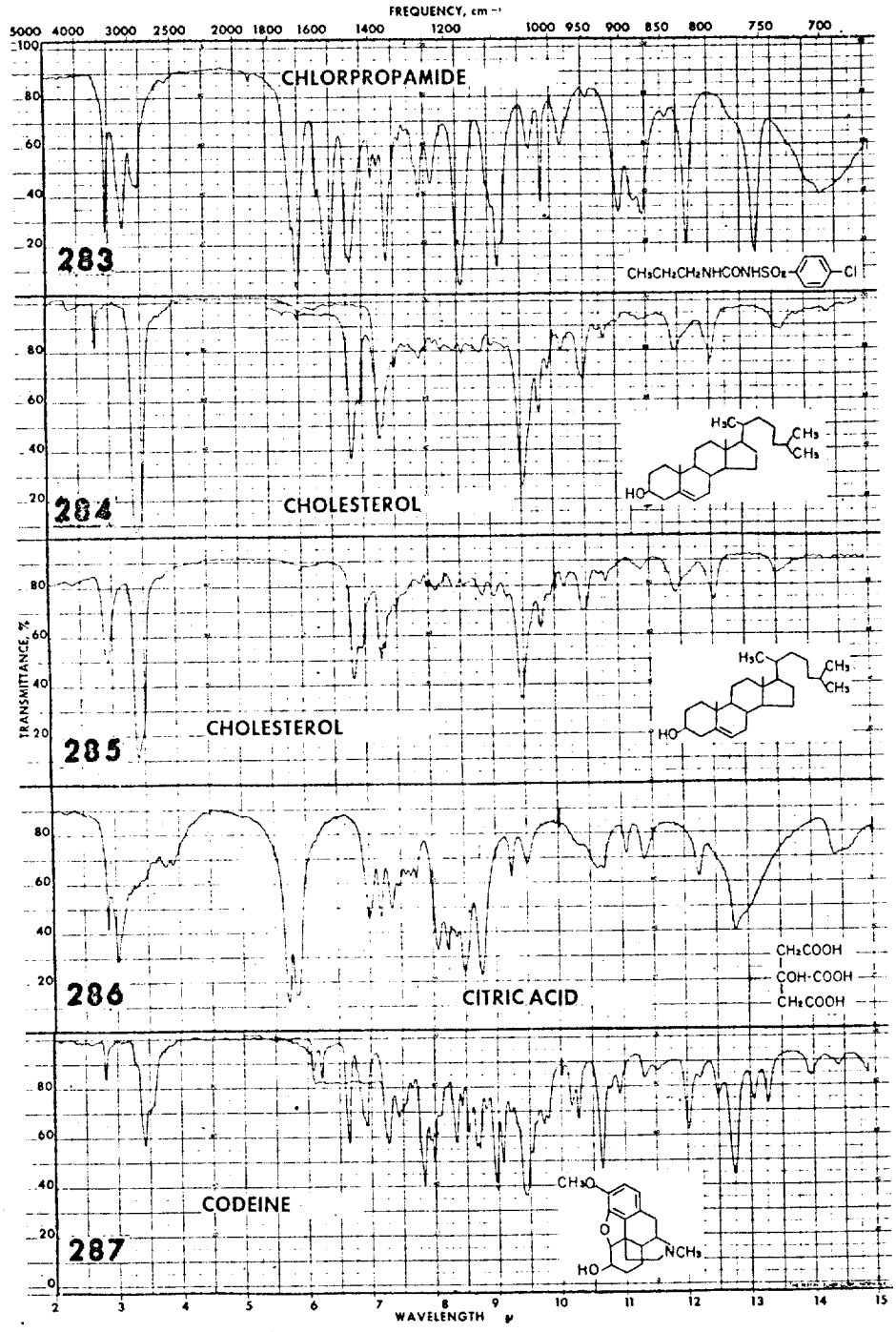

1000 950 900 850 800 750 700
MINE MALEATE
HCCOO⁻
HCCOOH
CHCH₂CH₂N(CH₃)₂
DROCHLORIDE
HCl
CH₃
CH₃CH₂CH₂CH₂OH
10 11 12 13 14 15

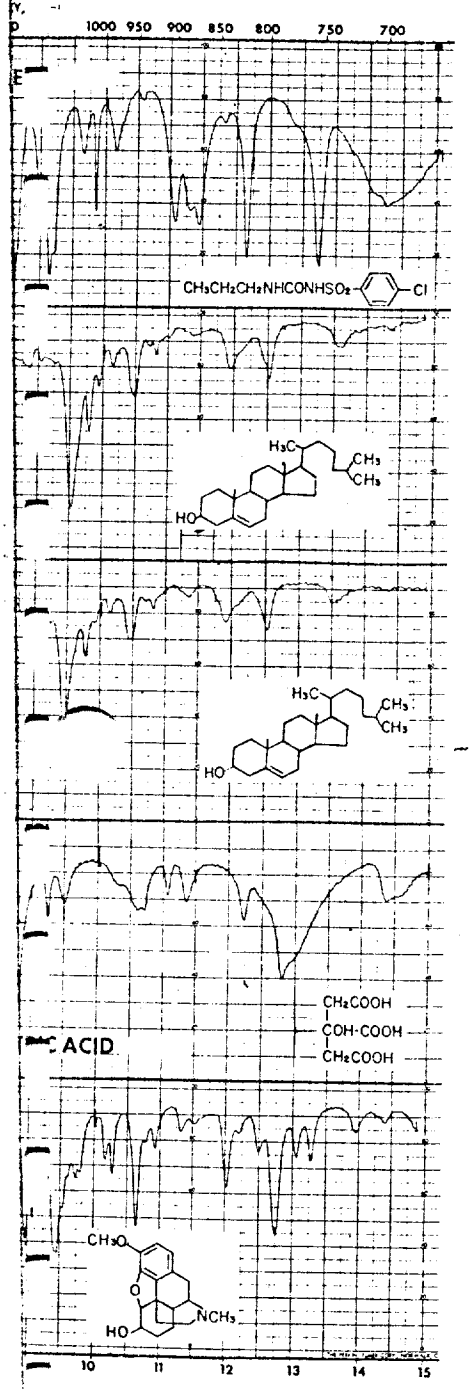

FREQUENCY, cm
4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
268
SEC-BUTYL ALCOHOL
CH₃CHCH₂CH₃
OH
269
BUTYLATED HYDROXY TOLUENE
270
CAFFEINE
271
CALCIUM CYANAMIDE
CaCN₂
272
CAMPHOR
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

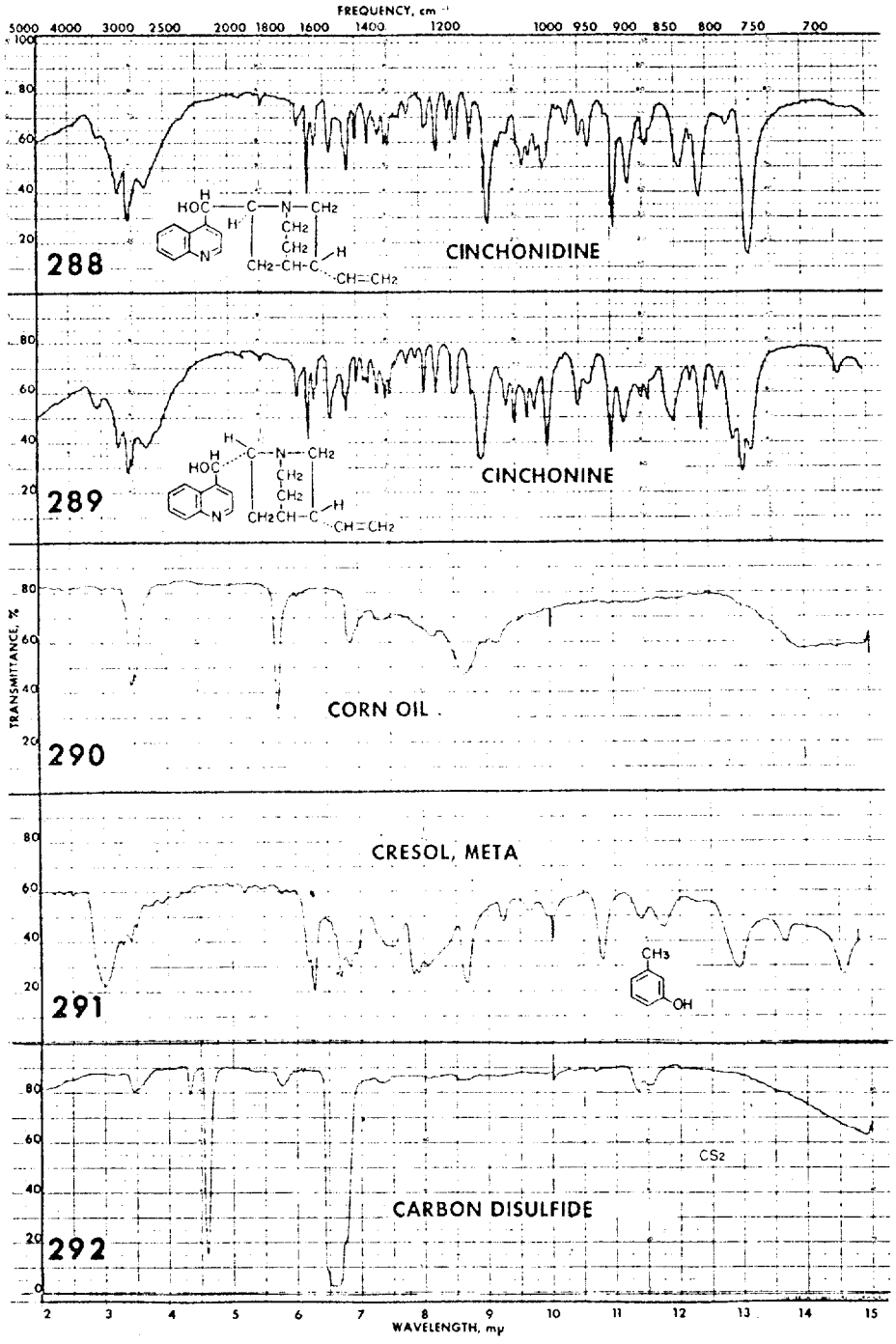

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
273
CAMPHOR-10-SULFONIC ACID
CH₂SO₃H
O
CH₃
CH₃
ABSORBANCE
274
CARAMIPHEN ETHANEDISULFONATE
COOCH₂CH₂N(C₂H₅)₂
CH₂SO₃H
2 CH₂SO₃H
275
CARBIMAZOLE
CH₃
SCOOC₂H₅
TRANSMITTANCE, %
276
CARBOCAINE HYDROCHLORIDE
Cl⁻
CH₃
CONH
CH₃
CH₃
277
CARDRASE
C₂H₅O
SO₂NH₂
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

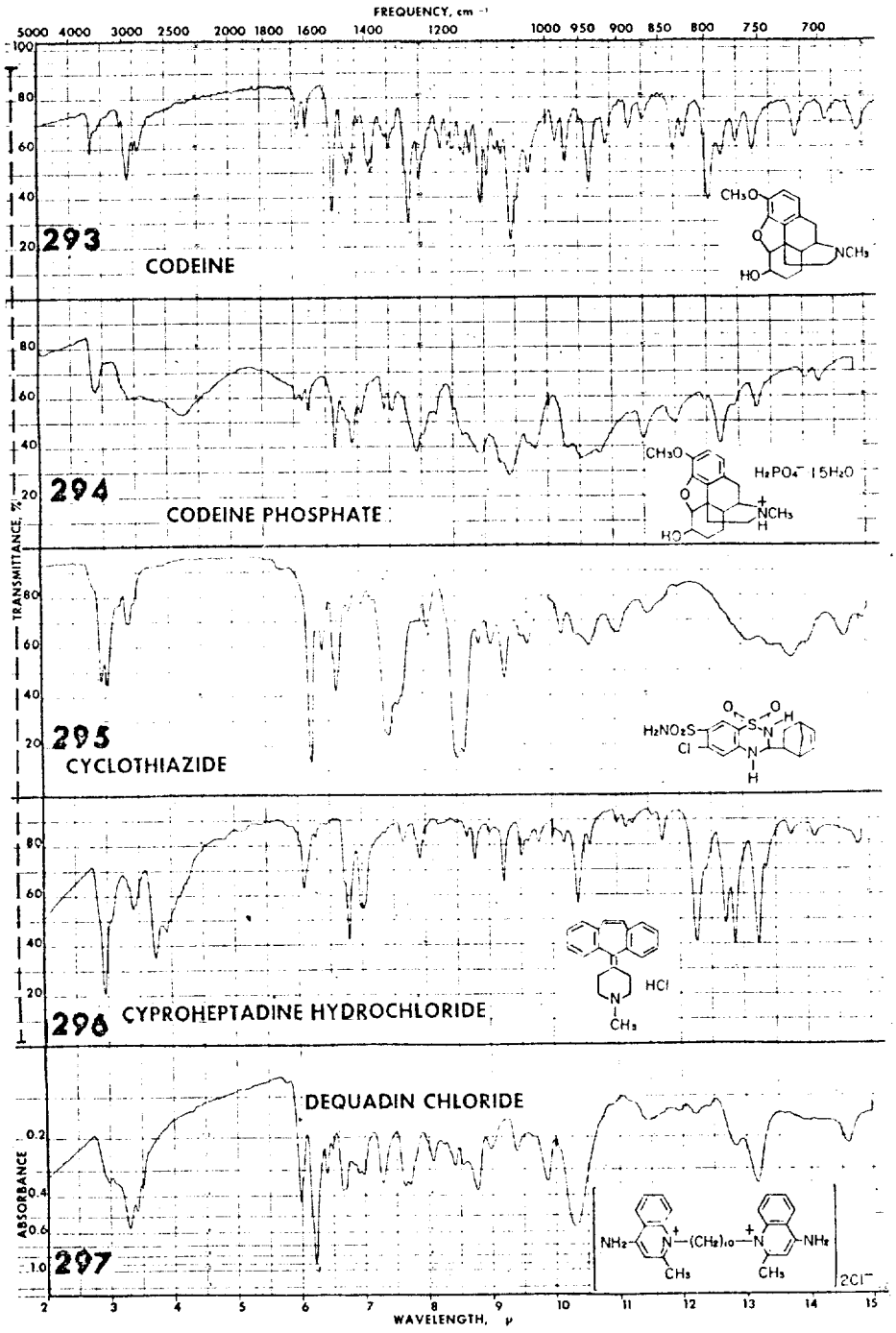

1000 950 900 850 800 750 700
CH2SO3H
O
CH3
C
CH3
COOCH2CH2N(C2H5)2
CH2SO3H
2 CH2SO3H
CH3
N
SCOOC2H5
N
IMAZOLE
CHLORIDE
Cl⁻
CONH
C2H5O
S
SO2NH2
N
9 10 11 12 13 14 15

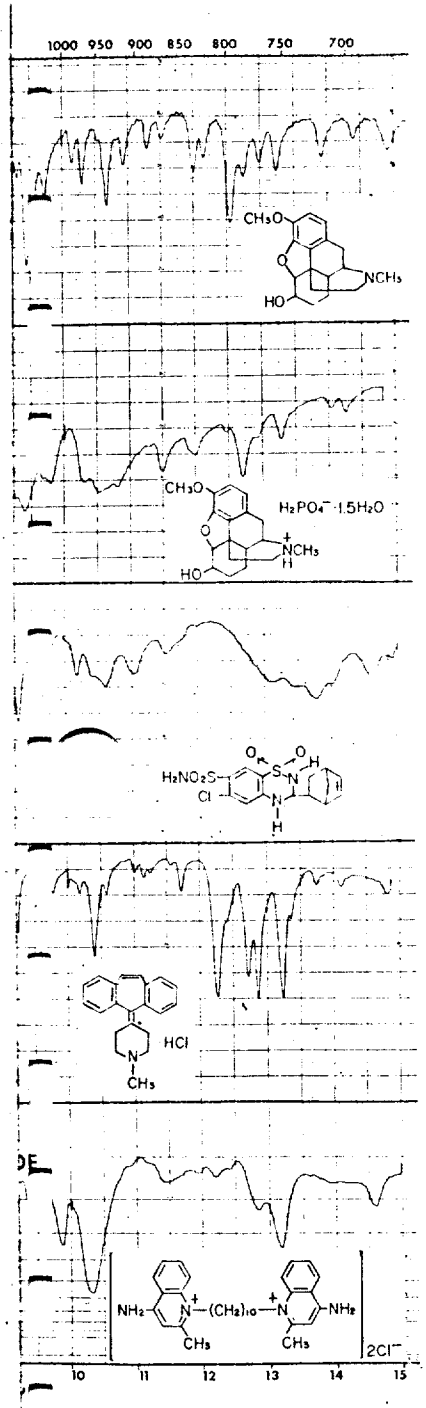

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
278
CASTOR OIL
279
CELLULOSE POWDER
(C6H10O5)n
280
CHLORDANTOIN
NH-C
O
Cl
NSC-Cl
Cl
CH3CH2CH2CH2CH-CH-C
C2H5
O
ABSORBANCE
281
CHLORHEXIDINE DIHYDROCHLORIDE
Cl
NHCNHCNH(CH2)6NHCNHCNH
NH NH 2HCl NH NH
Cl
282
CHLOROPROCAINE HYDROCHLORIDE
NH2
O
COCH2CH2N
C2H5
HCl
Cl
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

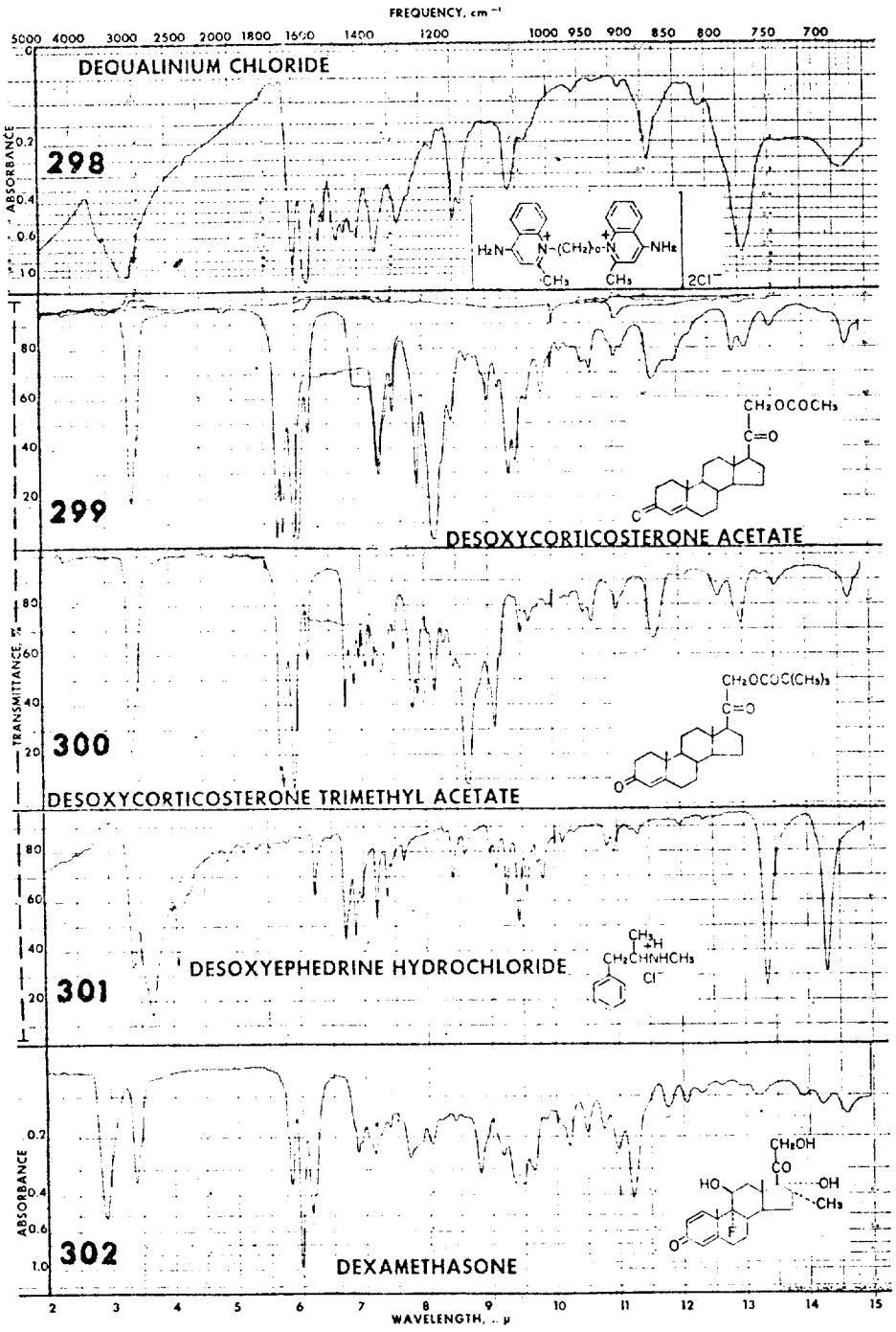

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
CHLORPROPAMIDE
283
CH3CH2CH2NHCONHSO2
Cl
284
CHOLESTEROL
HO
285
CHOLESTEROL
TRANSMITTANCE, %
286
CITRIC ACID
CH2COOH
COH-COOH
CH2COOH
CODEINE
287
CH3O
NCH3
HO
WAVELENGTH μ

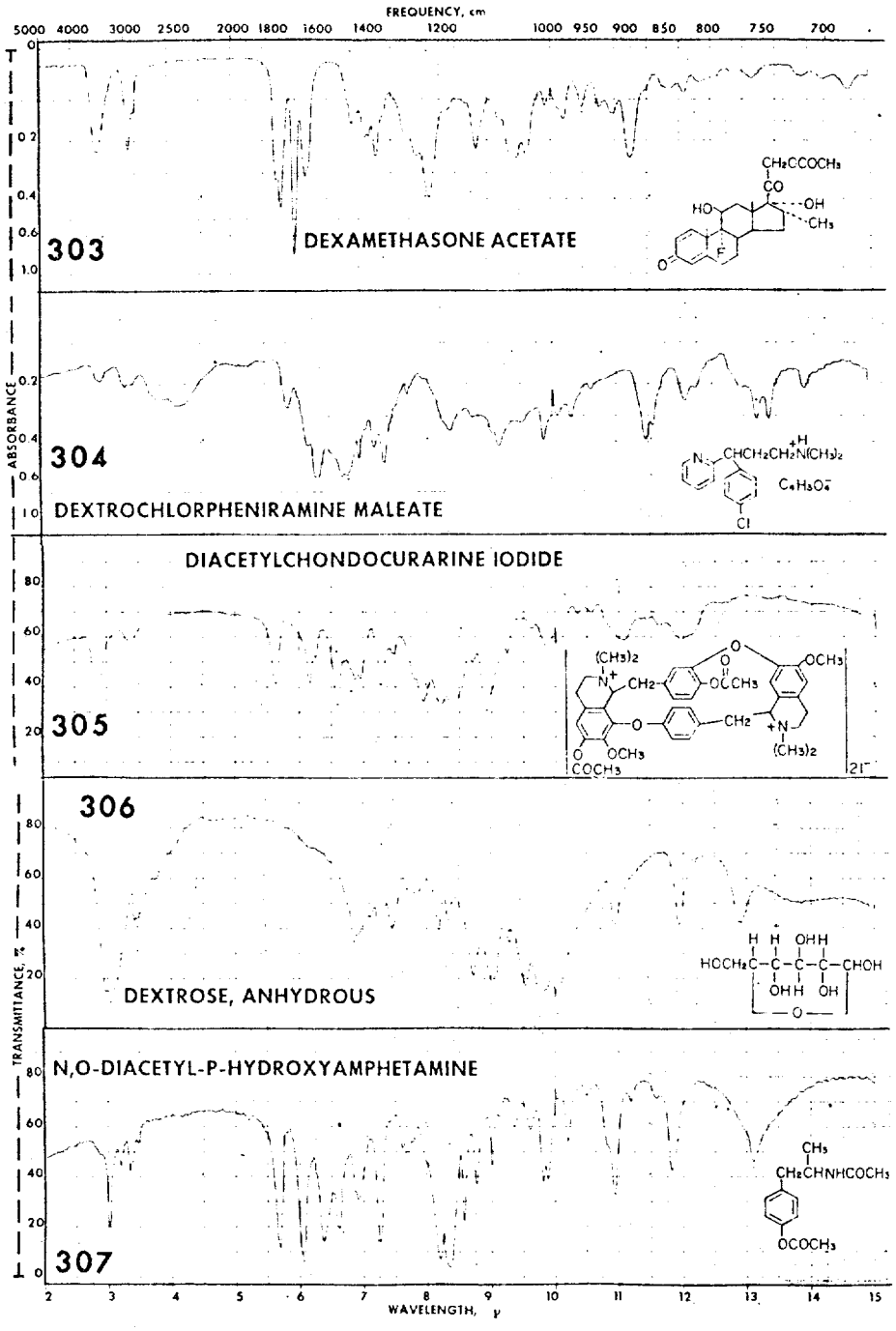

1000 950 900 850 800 750 700
CH3CH2CH2NHCONHSO2-
Cl
HO
ACID
CH2COOH
COH·COOH
CH2COOH
CH3O
NCH3
HO
10 11 12 13 14 15

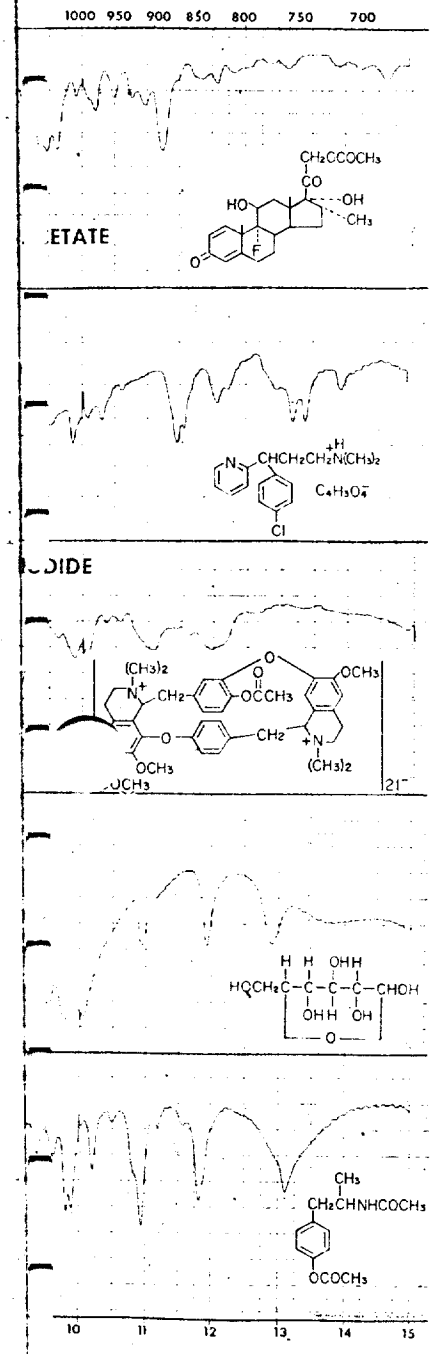

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
288
CINCHONIDINE
289
CINCHONINE
290
CORN OIL
291
CRESOL, META
292
CARBON DISULFIDE
CS2
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, mμ

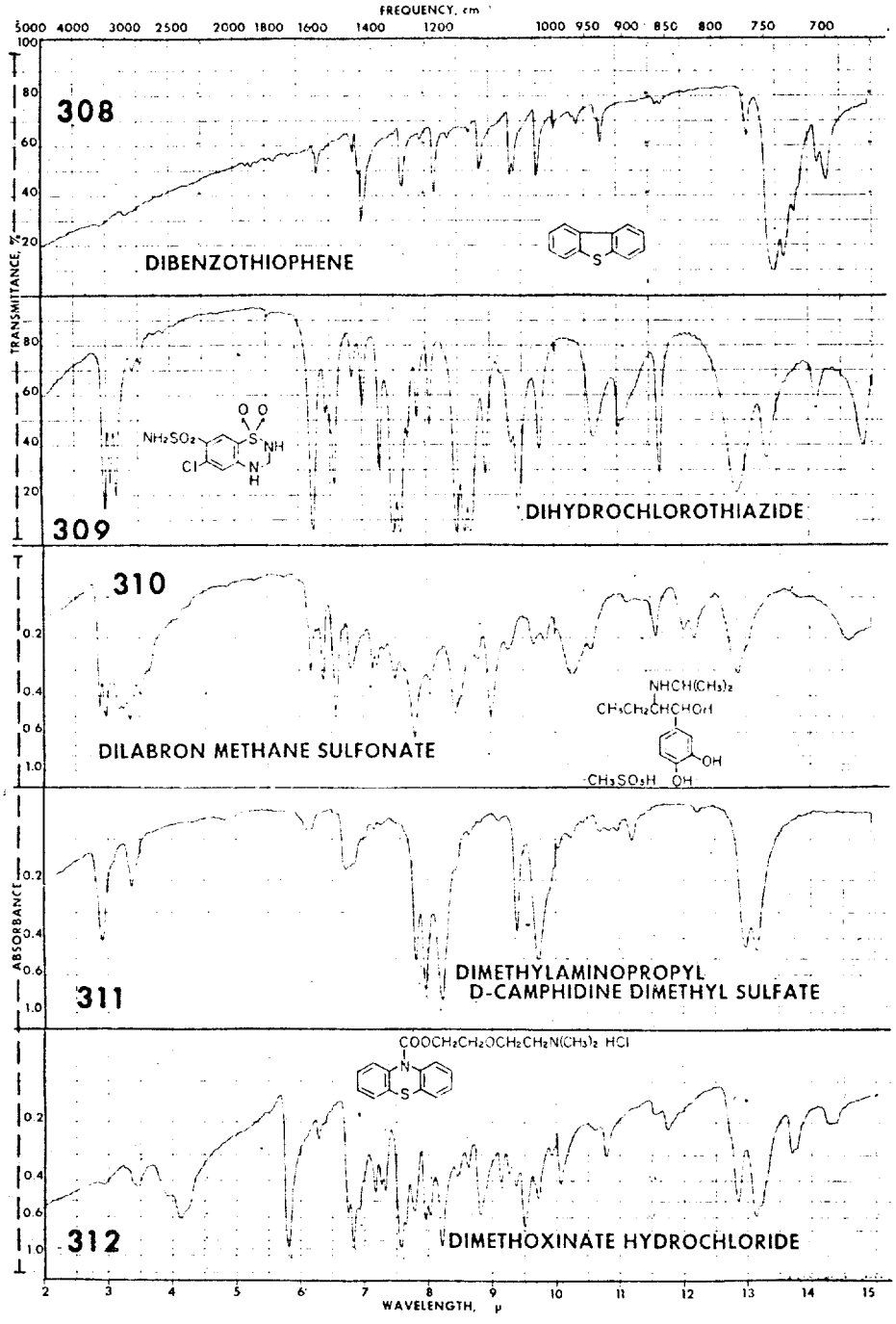

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
293
CODEINE
294
CODEINE PHOSPHATE
295
CYCLOTHIAZIDE
296
CYPROHEPTADINE HYDROCHLORIDE
297
DEQUADIN CHLORIDE
ABSORBANCE
WAVELENGTH, μ

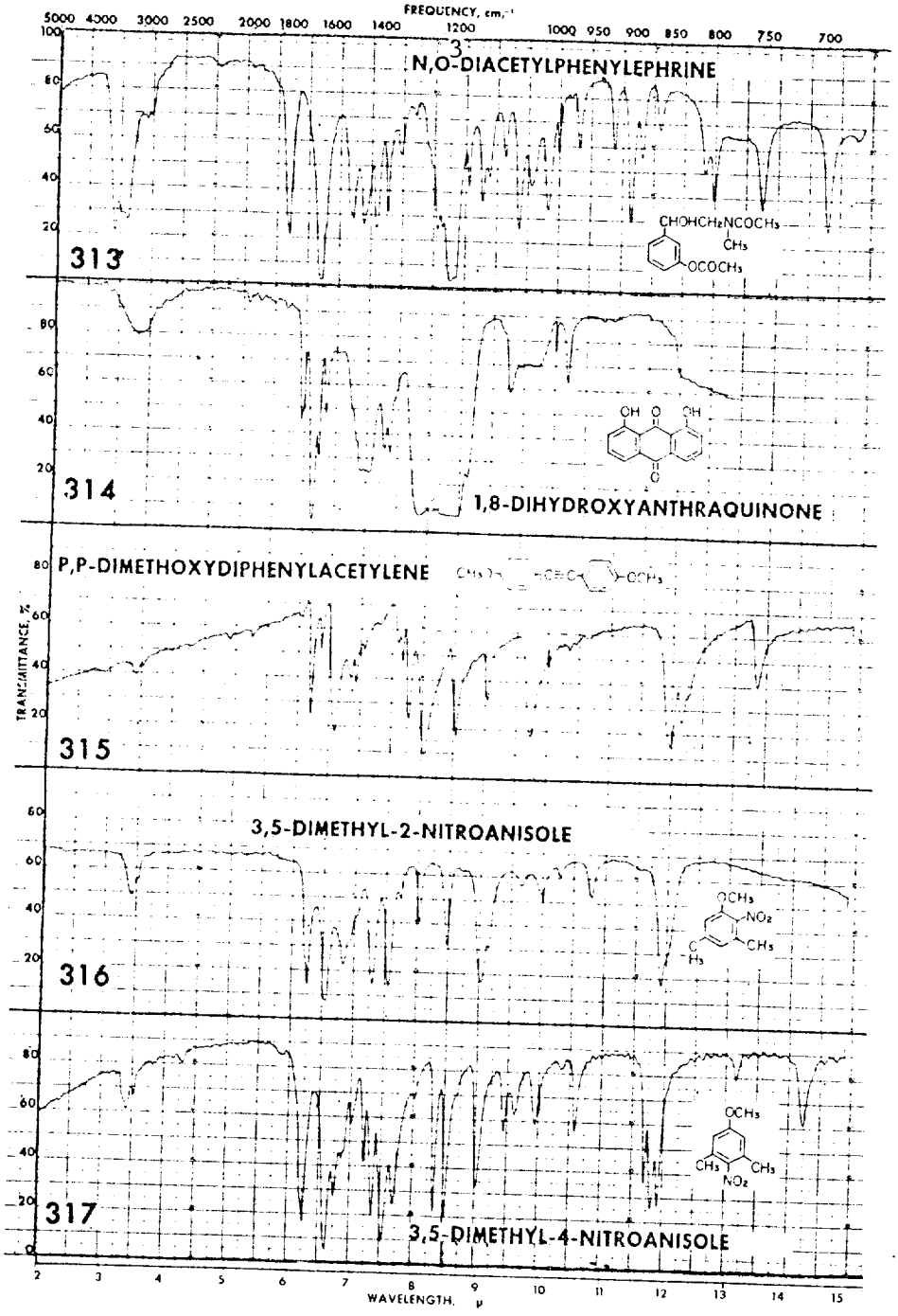

1000 950 900 850 800 750 700
CH3O
NCH3
HO
H2PO4− 1.5H2O
H2NO2S
Cl
HCl
2Cl−
10 11 12 13 14 15

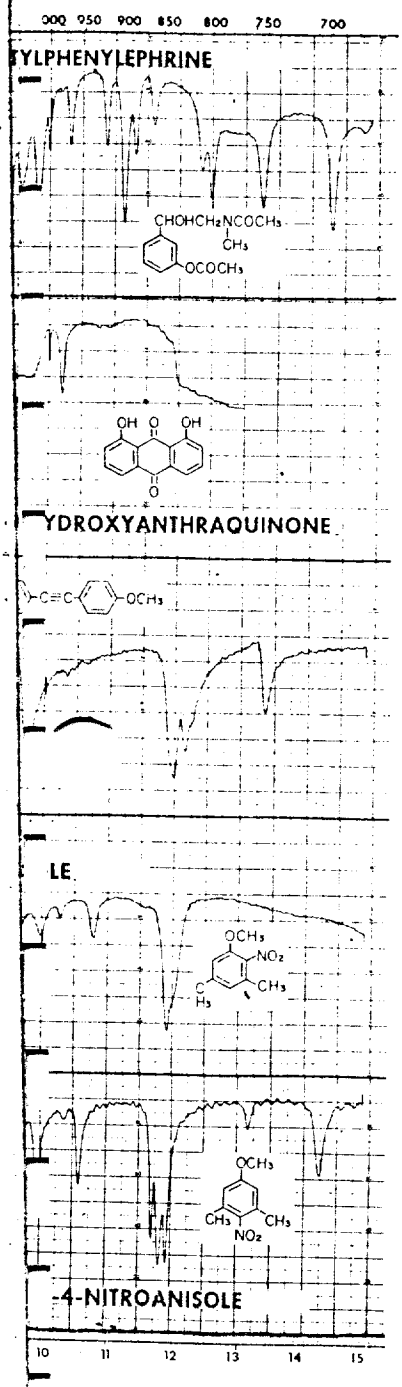

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
DEQUALINIUM CHLORIDE
298
2Cl−
TRANSMITTANCE, %
299
CH2OCOCH3
C=O
DESOXYCORTICOSTERONE ACETATE
300
CH2OCOC(CH3)3
DESOXYCORTICOSTERONE TRIMETHYL ACETATE
301
DESOXYEPHEDRINE HYDROCHLORIDE
Cl−
ABSORBANCE
302
CH2OH
CO
HO
OH
CH3
F
DEXAMETHASONE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, .. μ

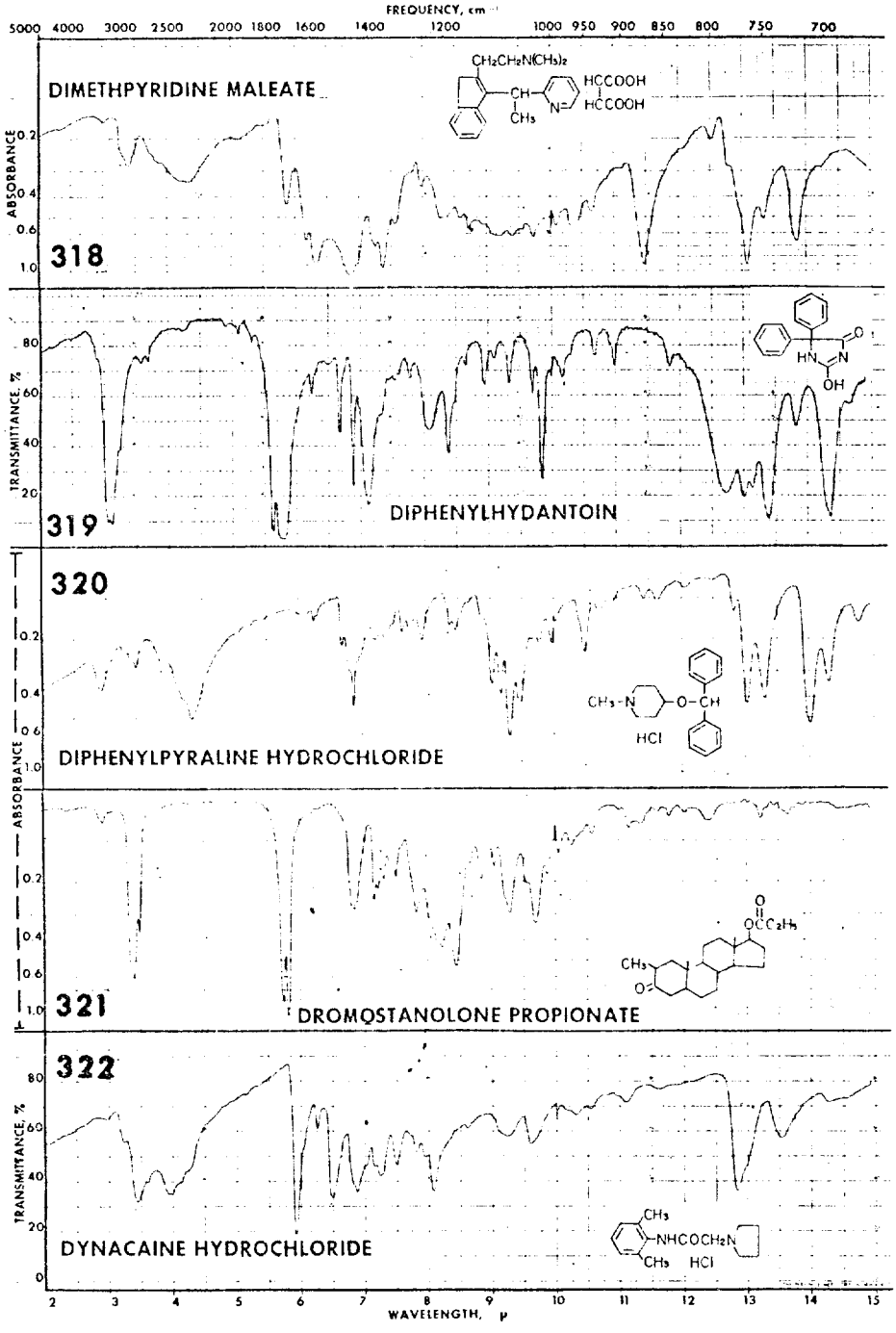

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
T
0
0.2
0.4
0.6
1.0
303
DEXAMETHASONE ACETATE
ABSORBANCE
0.2
0.4
0.6
1.0
304
DEXTROCHLORPHENIRAMINE MALEATE
DIACETYLCHONDOCURARINE IODIDE
80
60
40
20
305
306
TRANSMITTANCE, %
80
60
40
20
DEXTROSE, ANHYDROUS
N,O-DIACETYL-P-HYDROXYAMPHETAMINE
80
60
40
20
0
307
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

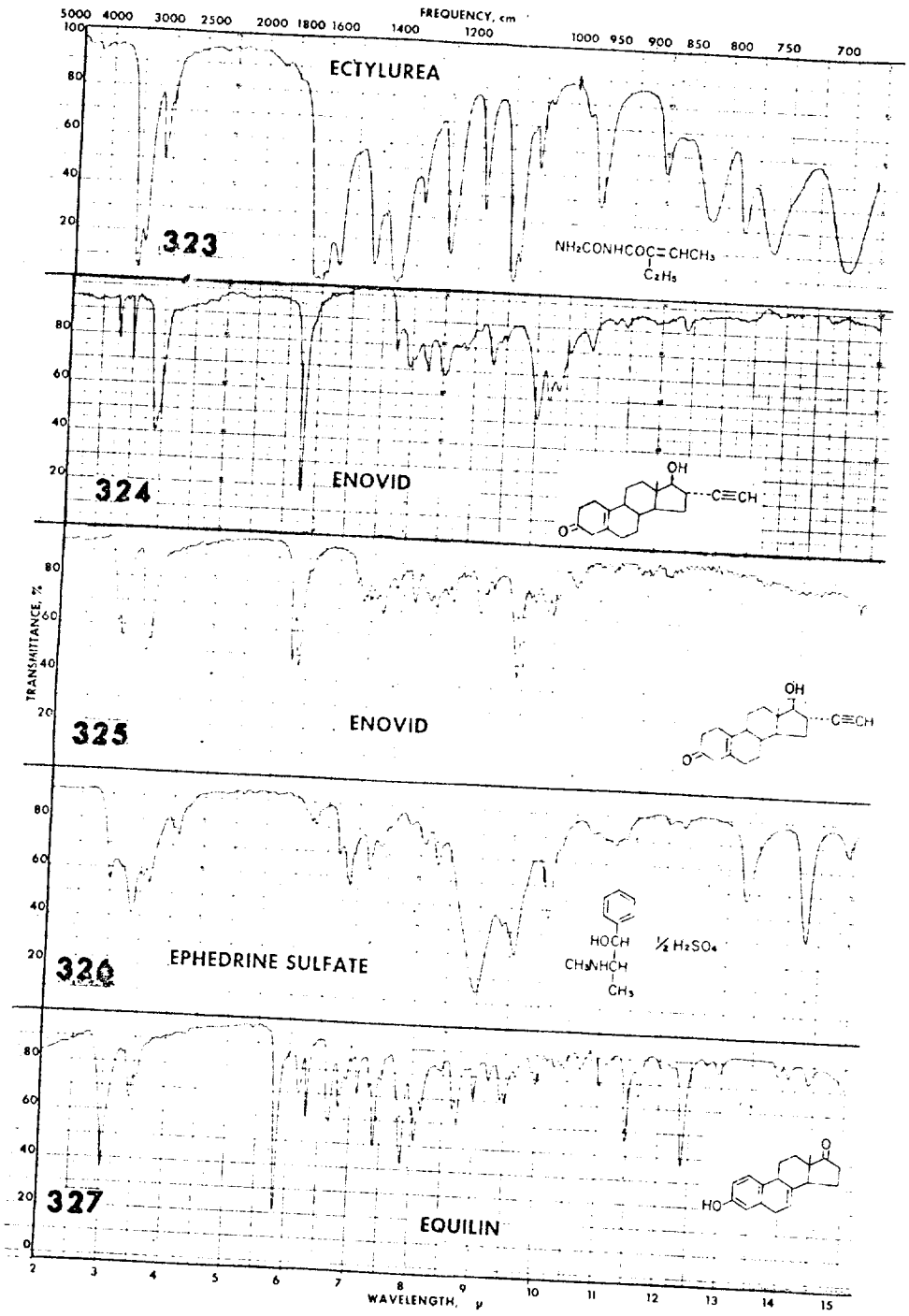

1000 950 900 850 800 750 700
ETATE
DIDE
10 11 12 13 14 15

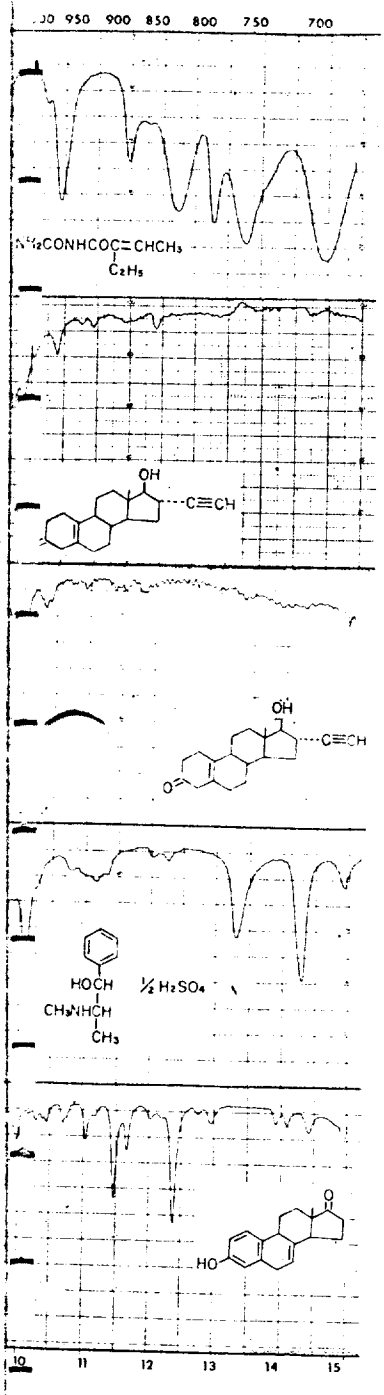

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
308
DIBENZOTHIOPHENE
309
DIHYDROCHLOROTHIAZIDE
310
DILABRON METHANE SULFONATE
ABSORBANCE
311
DIMETHYLAMINOPROPYL
D-CAMPHIDINE DIMETHYL SULFATE
312
DIMETHOXINATE HYDROCHLORIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

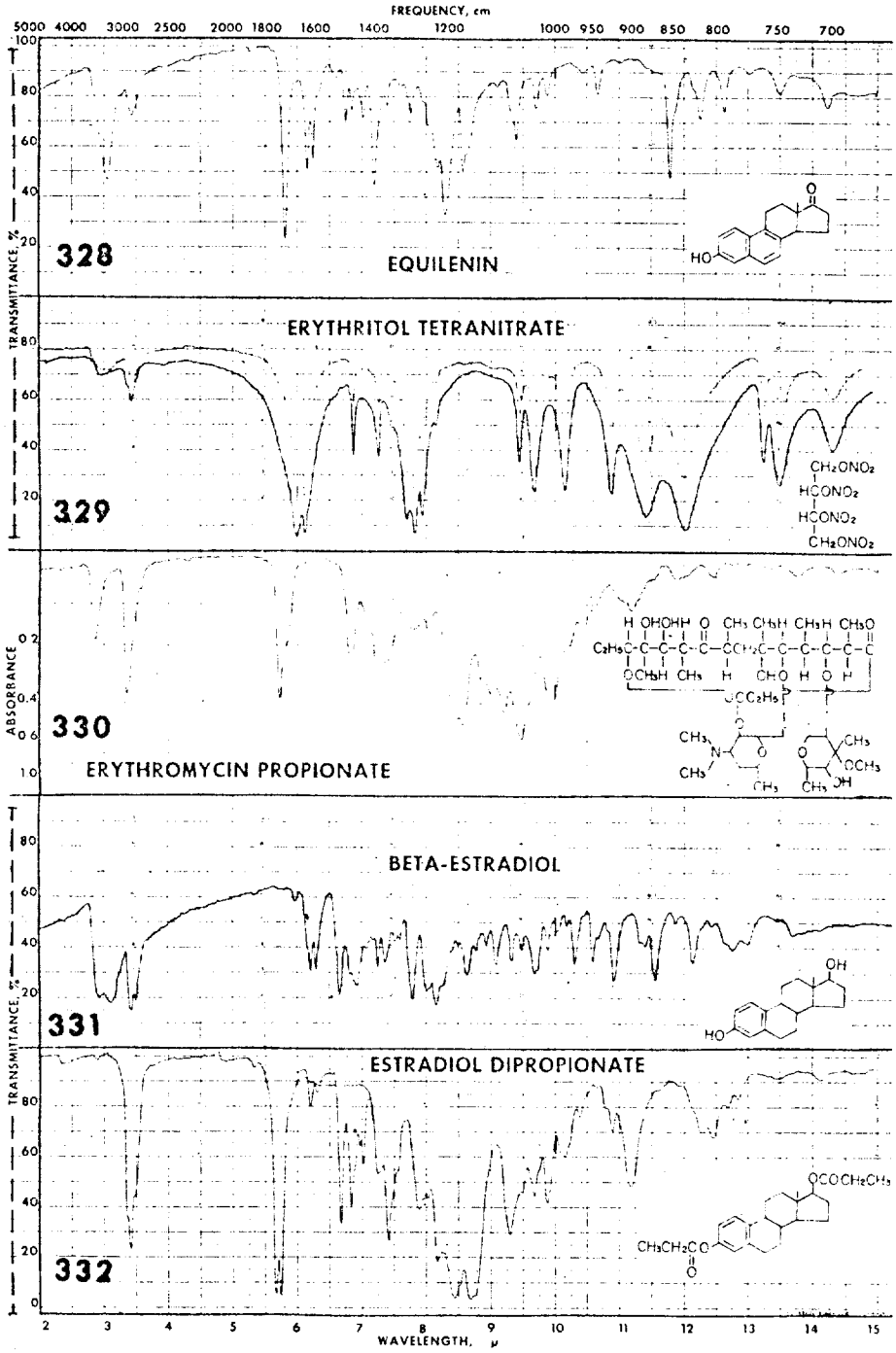

FREQUENCY, cm.⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
313
N,O-DIACETYLPHENYLEPHRINE
CHOHCH₂NCOCH₃
CH₃
OCOCH₃
314
1,8-DIHYDROXYANTHRAQUINONE
OH O OH
O
315
P,P-DIMETHOXYDIPHENYLACETYLENE
CH₃O
OCH₃
TRANSMITTANCE, %
316
3,5-DIMETHYL-2-NITROANISOLE
OCH₃
NO₂
CH₃
CH₃
317
3,5-DIMETHYL-4-NITROANISOLE
OCH₃
CH₃
CH₃
NO₂
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

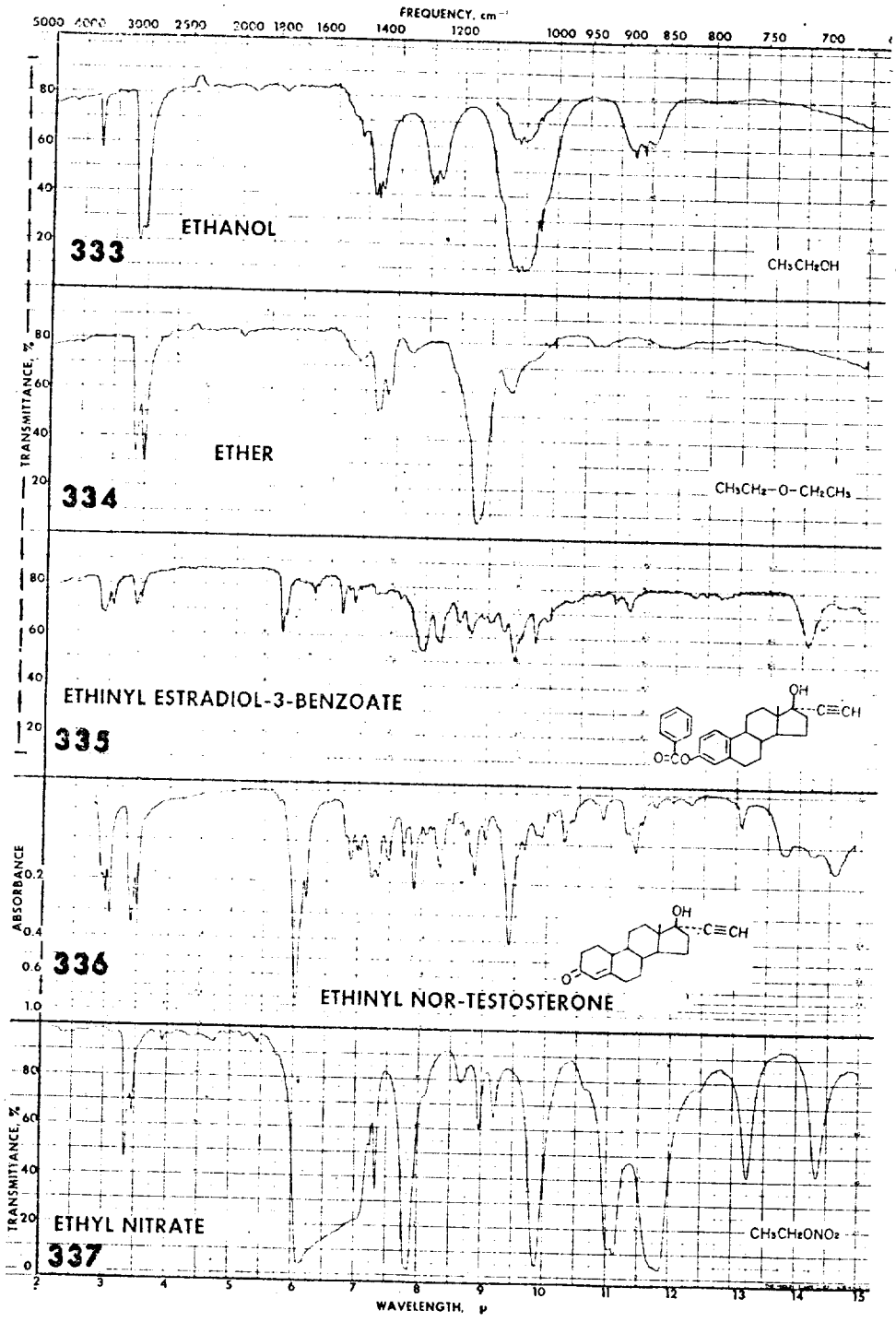

900 950 900 850 800 750 700
YLPHENYLEPHRINE
CHOHCH2NCOCH3
CH3
OCOCH3
OH O OH
O
YDROXYANTHRAQUINONE
C≡C
OCH3
LE
OCH3
NO2
CH3
CH3
OCH3
CH3
CH3
NO2
-4-NITROANISOLE
10 11 12 13 14 15

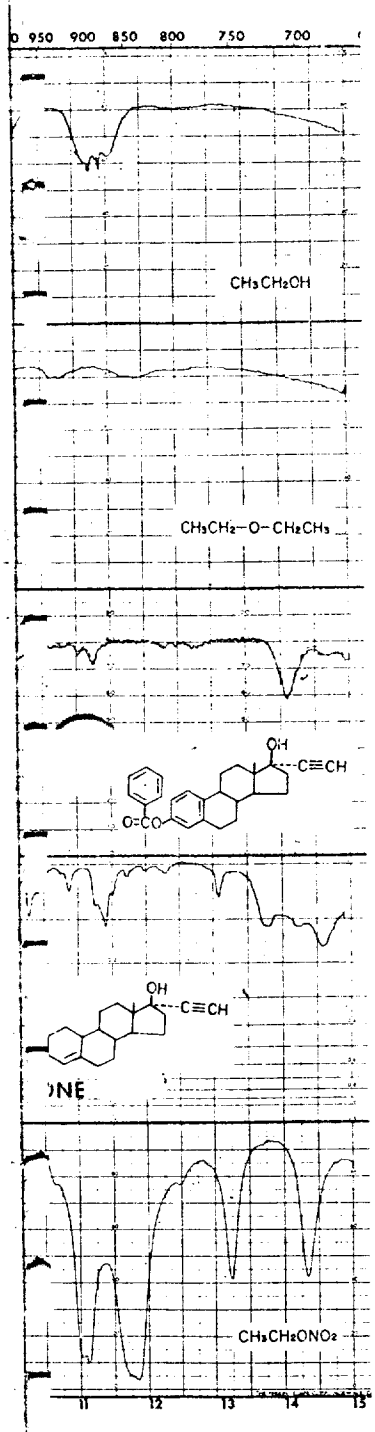

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
DIMETHPYRIDINE MALEATE
CH2CH2N(CH3)2
CH
CH3
N=
HCCOOH
HCCOOH
ABSORBANCE
0.2
0.4
0.6
1.0
318
TRANSMITTANCE, %
80
60
40
20
HN
N
OH
319
DIPHENYLHYDANTOIN
320
0.2
0.4
0.6
1.0
CH3-N
O-CH
HCl
DIPHENYLPYRALINE HYDROCHLORIDE
ABSORBANCE
0.2
0.4
0.6
1.0
O
OC2H5
CH3
O
321
DROMOSTANOLONE PROPIONATE
322
TRANSMITTANCE, %
80
60
40
20
0
CH3
NHCOCH2N
CH3
HCl
DYNACAINE HYDROCHLORIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

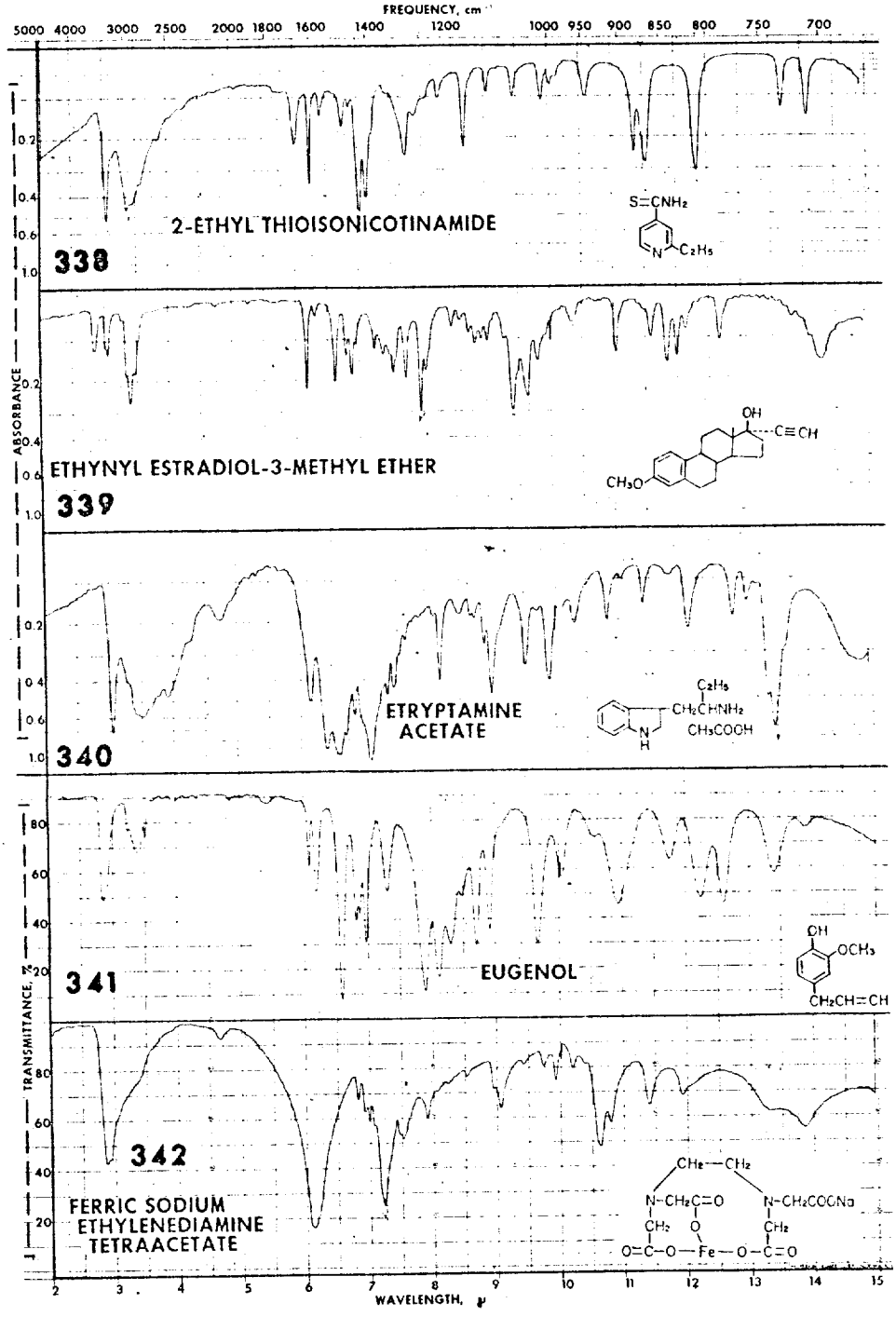

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ECTYLUREA
323
NH2CONHCOC=CHCH3
C2H5
324
ENOVID
OH
C≡CH
O
TRANSMITTANCE, %
325
ENOVID
OH
C≡CH
O
326
EPHEDRINE SULFATE
HOCH
CH3NHCH
CH3
½ H2SO4
327
EQUILIN
HO
O
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

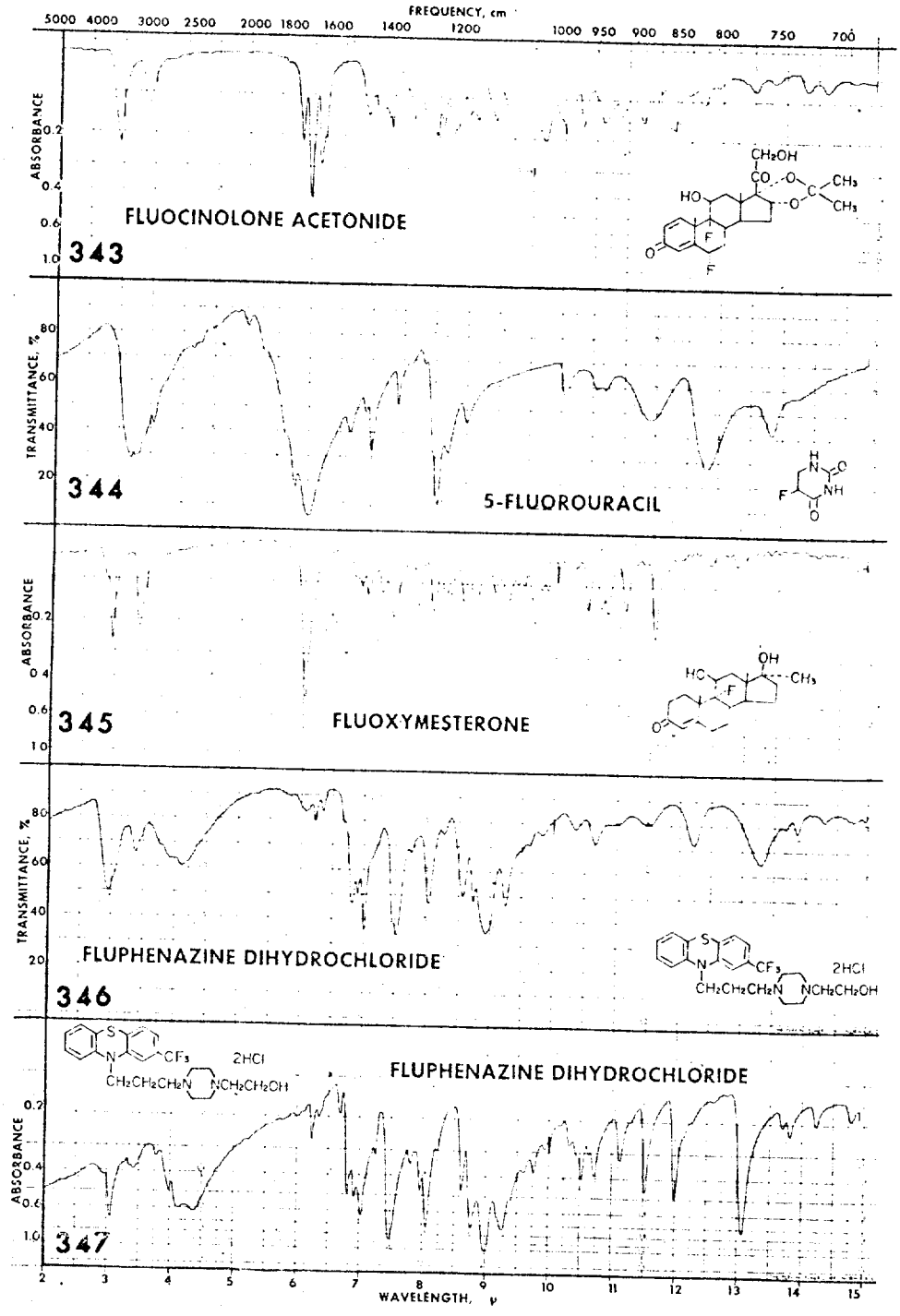

...00 950 900 850 800 750 700
...½CONHCOC=CHCH₃
C₂H₅
OH
C≡CH
OH
C≡CH
O
HOCH
CH₃NHCH
CH₃
½ H₂SO₄
HO
O
10 11 12 13 14 15

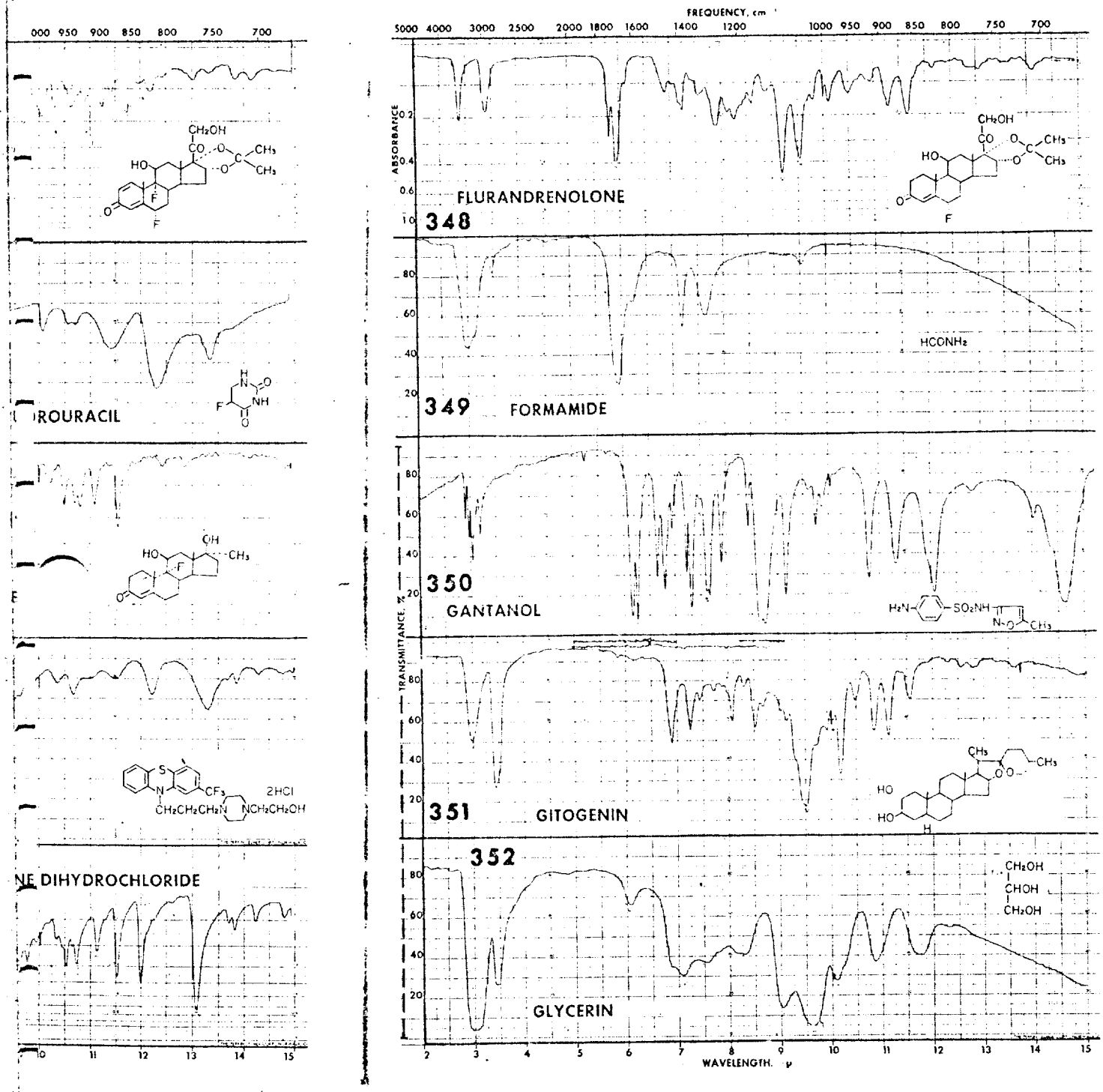

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
328
EQUILENIN
HO
O
ERYTHRITOL TETRANITRATE
329
CH₂ONO₂
HCONO₂
HCONO₂
CH₂ONO₂
ABSORBANCE
330
ERYTHROMYCIN PROPIONATE
BETA-ESTRADIOL
331
HO
OH
ESTRADIOL DIPROPIONATE
332
OCOCH₂CH₃
CH₃CH₂CO
O
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

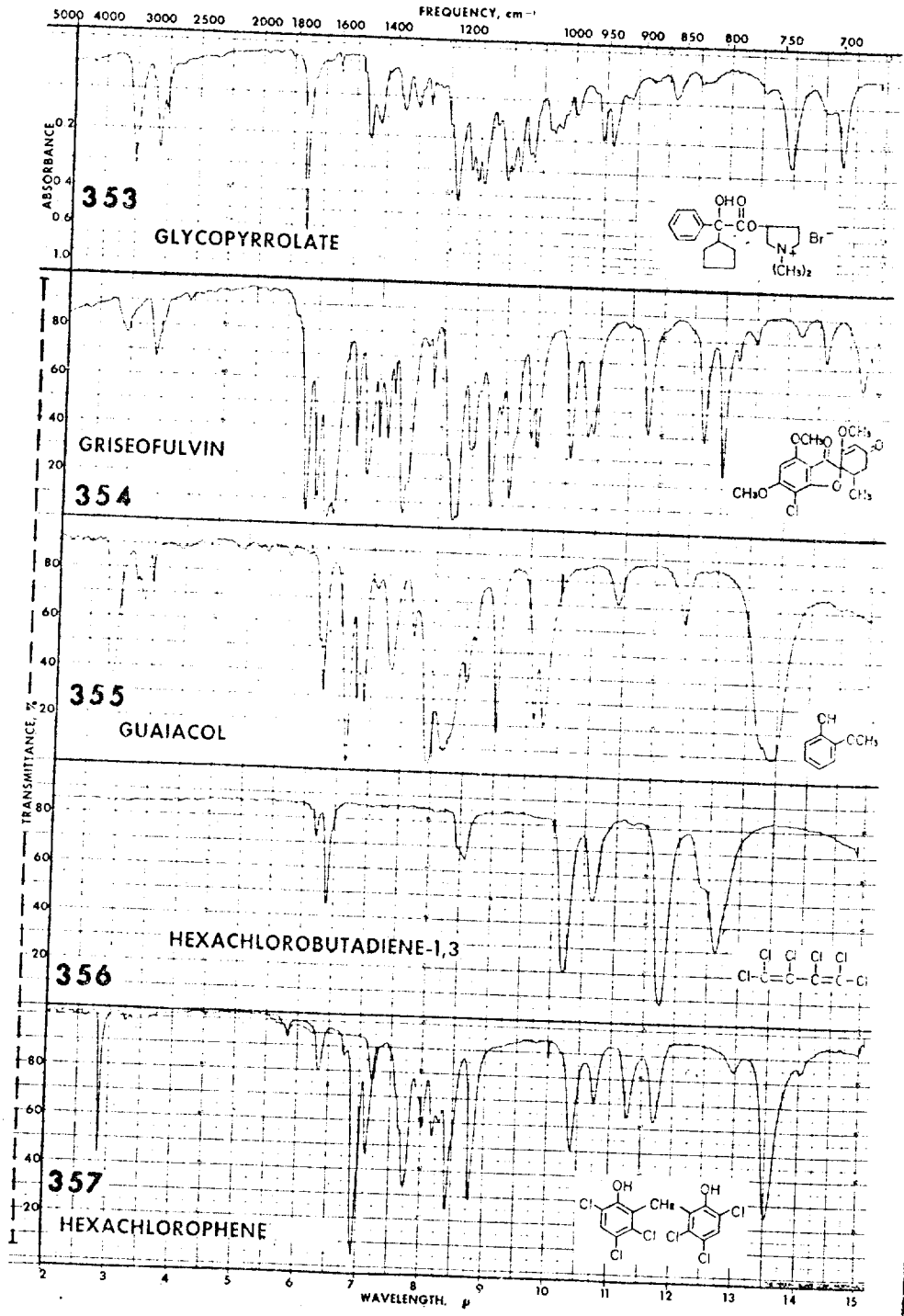

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
ETHANOL
333
CH₃CH₂OH
ETHER
334
CH₃CH₂—O—CH₂CH₃
ETHINYL ESTRADIOL-3-BENZOATE
335
OH
C≡CH
O=C-O
ABSORBANCE
336
ETHINYL NOR-TESTOSTERONE
OH
C≡CH
O
TRANSMITTANCE, %
ETHYL NITRATE
337
CH₃CH₂ONO₂
WAVELENGTH, μ

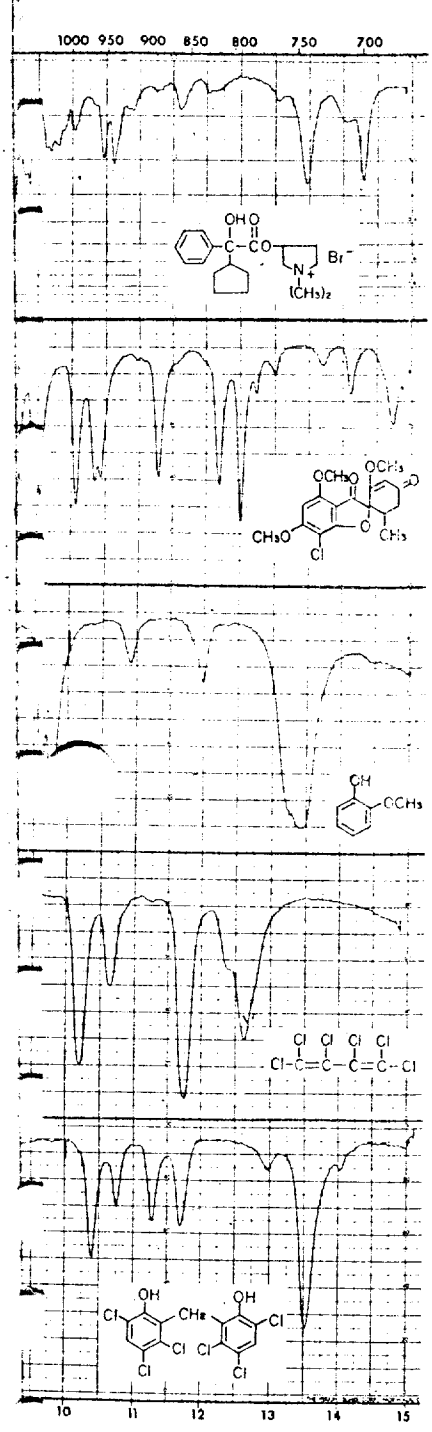

950 900 850 800 750 700
CH3CH2OH
CH3CH2—O—CH2CH3
OH
C≡CH
O=CO
ONE
OH
C≡CH
CH3CH2ONO2
11 12 13 14 15

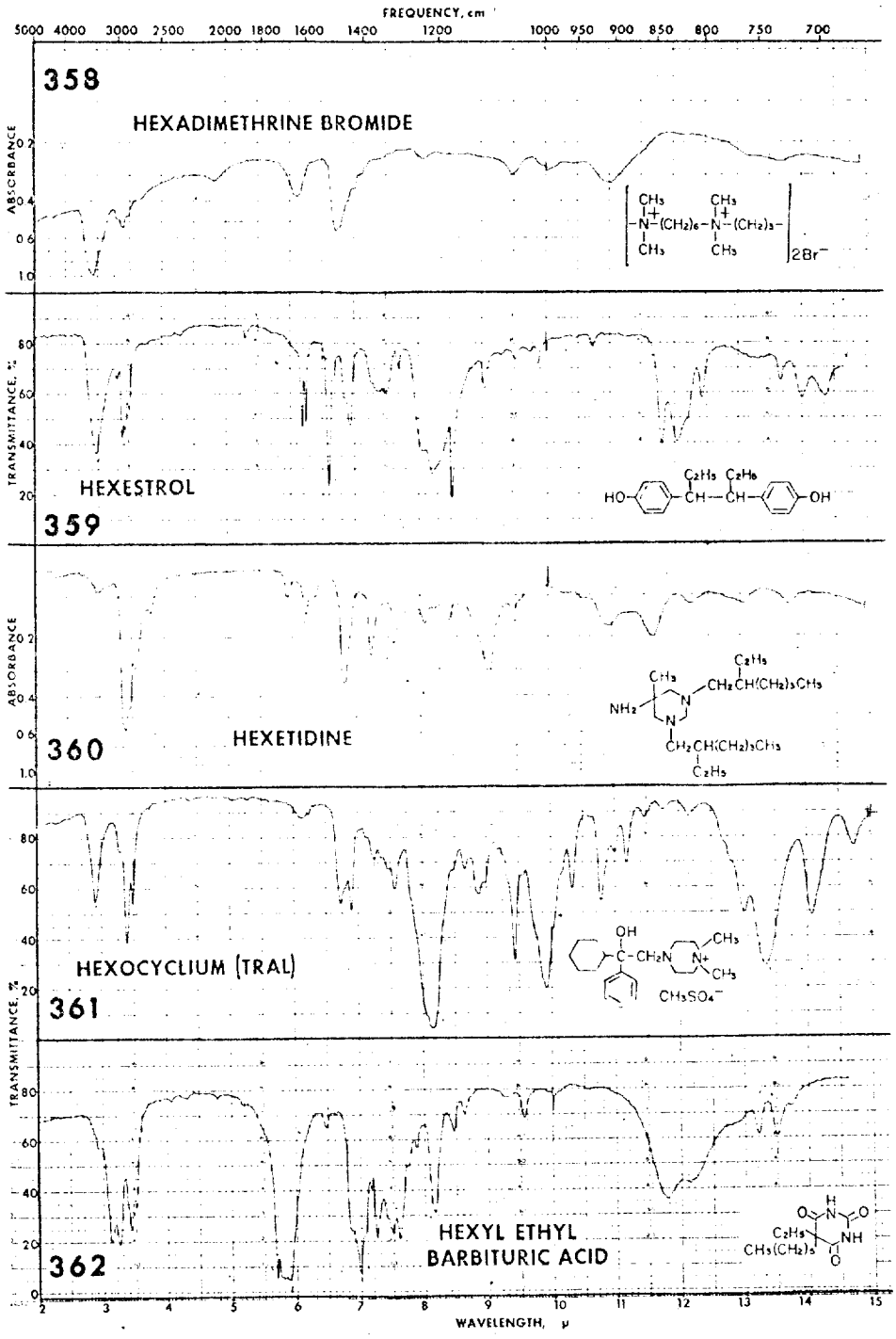

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
0.2
0.4
0.6
1.0
2-ETHYL THIOISONICOTINAMIDE
338
S=CNH2
N
C2H5
ETHYNYL ESTRADIOL-3-METHYL ETHER
339
OH
C≡CH
CH3O
ETRYPTAMINE ACETATE
340
C2H5
CH2CHNH2
N
H
CH3COOH
TRANSMITTANCE, %
80
60
40
20
EUGENOL
341
OH
OCH3
CH2CH=CH
342
FERRIC SODIUM ETHYLENEDIAMINE TETRAACETATE
CH2—CH2
N-CH2C=O
N-CH2COONa
CH2
O
CH2
O=C—O—Fe—O—C=O
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

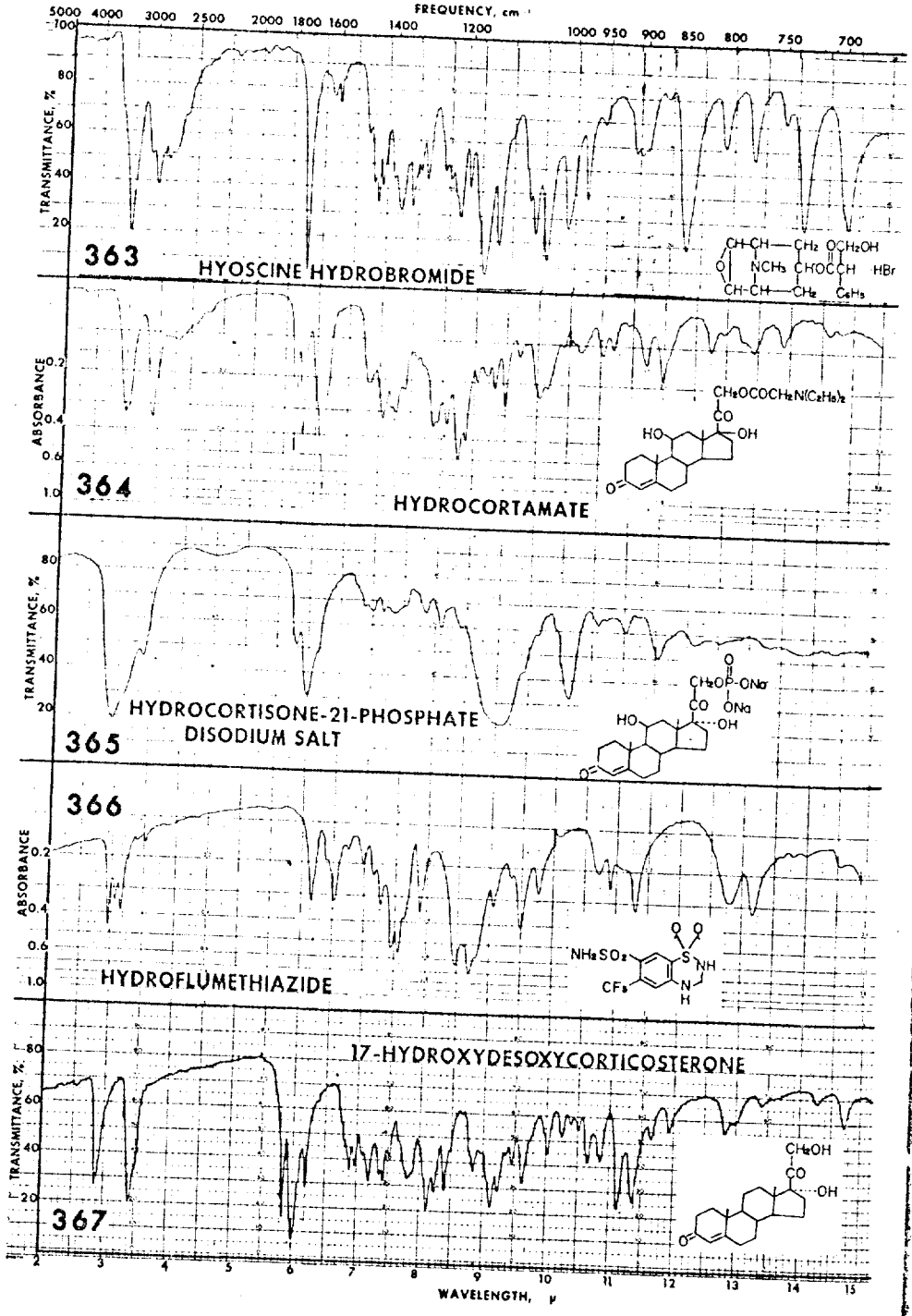

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
FLUOCINOLONE ACETONIDE
343
TRANSMITTANCE, %
344
5-FLUOROURACIL
ABSORBANCE
345
FLUOXYMESTERONE
TRANSMITTANCE, %
FLUPHENAZINE DIHYDROCHLORIDE
346
2HCl
FLUPHENAZINE DIHYDROCHLORIDE
ABSORBANCE
347
WAVELENGTH, μ

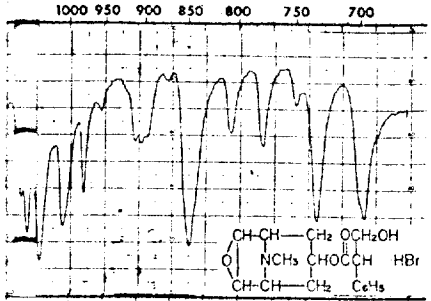

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
0.2
0.4
0.6
1.0
FLURANDRENOLONE
348
349
FORMAMIDE
HCONH₂
350
GANTANOL
TRANSMITTANCE, %
351
GITOGENIN
352
GLYCERIN
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15
000 950 900 850 800 750 700
ROURACIL
NE DIHYDROCHLORIDE
2HCl
10 11 12 13 14 15

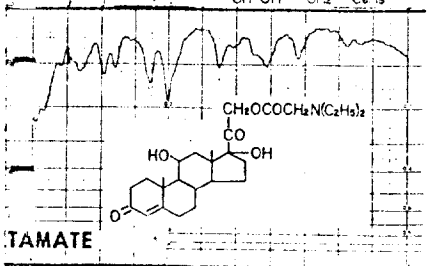

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
0.2
0.4
0.6
1.0
353
GLYCOPYRROLATE
OH
O
Br
(CH3)2
TRANSMITTANCE, %
80
60
40
20
GRISEOFULVIN
354
OCH3
CH3O
Cl
CH3
355
GUAIACOL
CH
OCH3
HEXACHLOROBUTADIENE-1,3
356
Cl
HEXACHLOROPHENE
357
OH
CH2
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

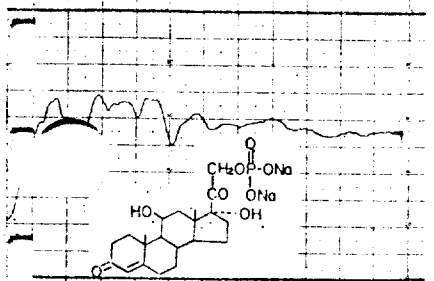

1000 950 900 850 800 750 700
10 11 12 13 14 15

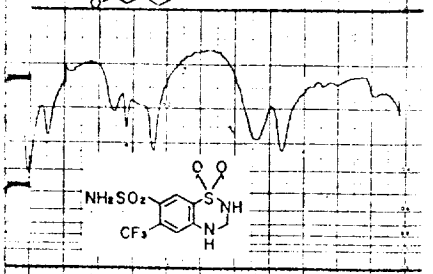

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
358
HEXADIMETHRINE BROMIDE
ABSORBANCE
TRANSMITTANCE, %
HEXESTROL
359
360
HEXETIDINE
HEXOCYCLIUM (TRAL)
361
362
HEXYL ETHYL BARBITURIC ACID
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

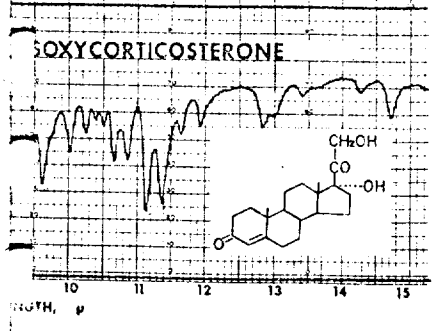

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
363
HYOSCINE HYDROBROMIDE
ABSORBANCE
364
HYDROCORTAMATE
TRANSMITTANCE, %
365
HYDROCORTISONE-21-PHOSPHATE
DISODIUM SALT
ABSORBANCE
366
HYDROFLUMETHIAZIDE
TRANSMITTANCE, %
367
17-HYDROXYDESOXYCORTICOSTERONE
WAVELENGTH, μ

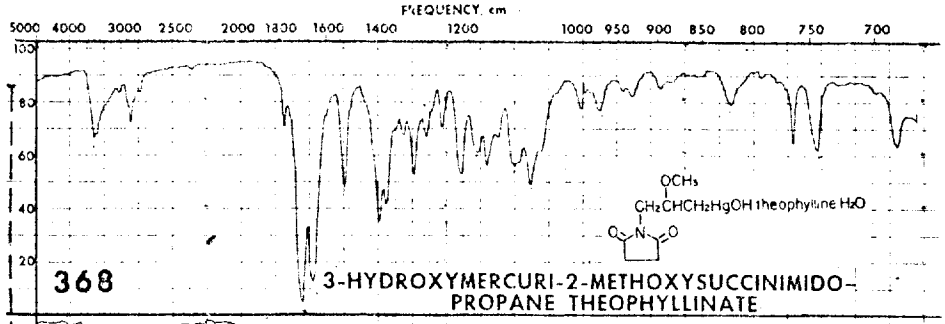

1000 950 900 850 800 750 700

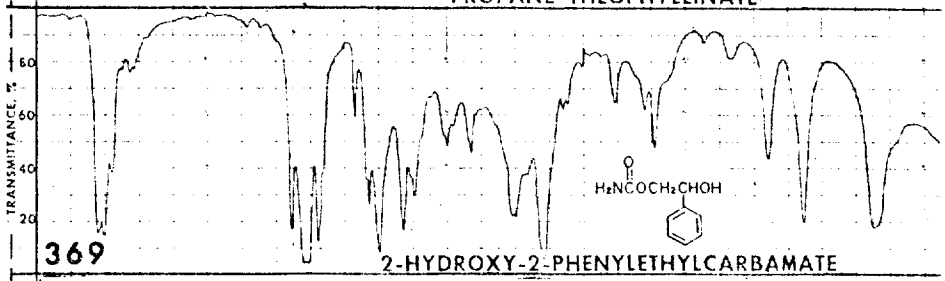

TAMATE

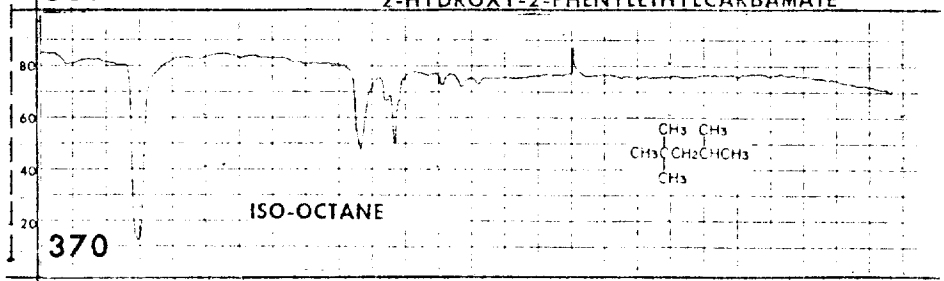


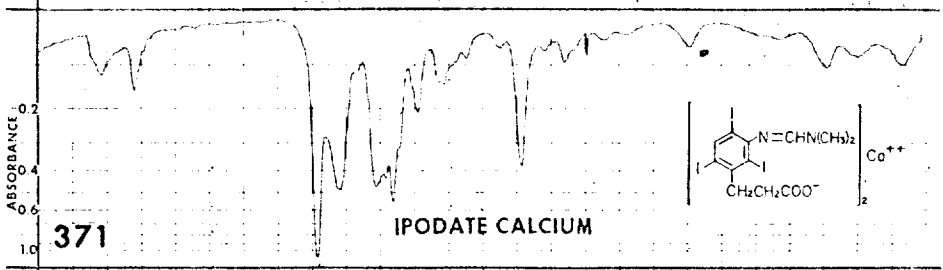


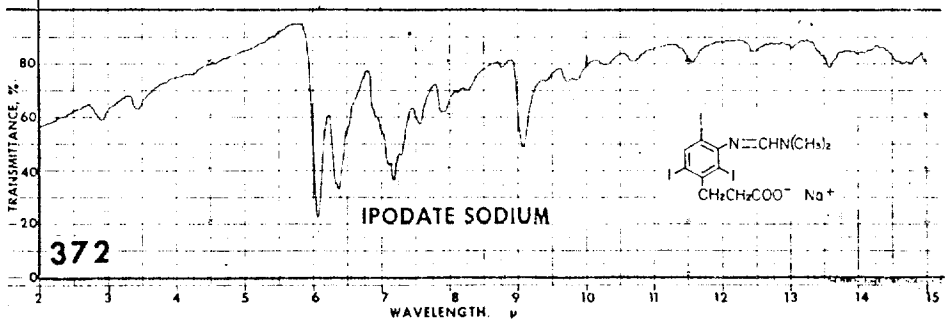

SOXYCORTICOSTERONE
10 11 12 13 14 15

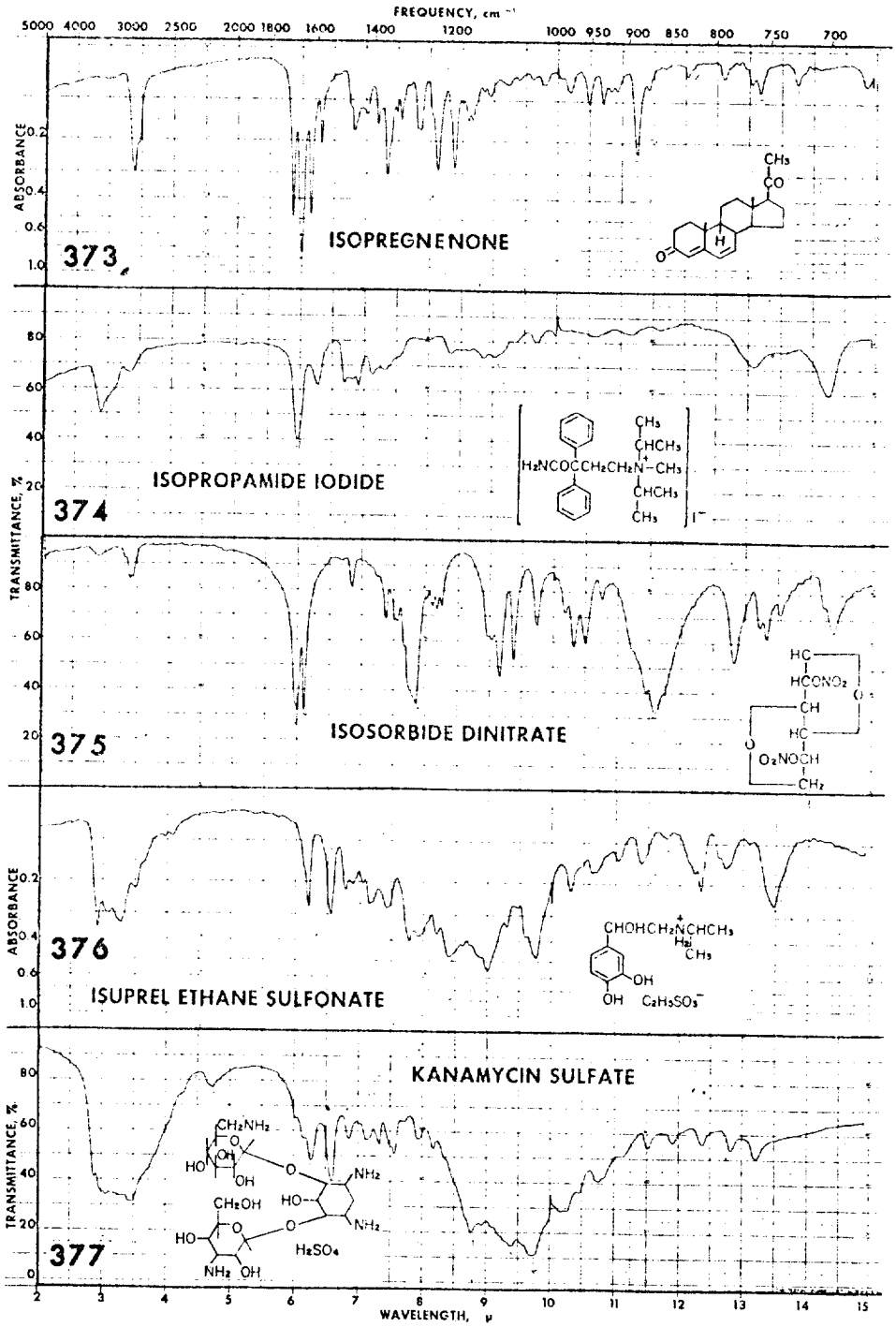

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
368
3-HYDROXYMERCURI-2-METHOXYSUCCINIMIDO-PROPANE THEOPHYLLINATE

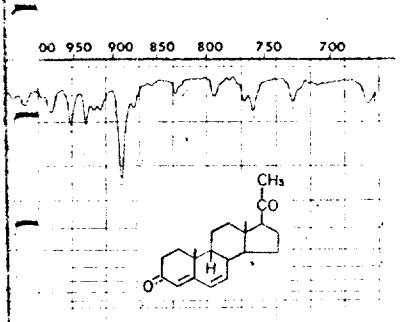

TRANSMITTANCE, %
369
2-HYDROXY-2-PHENYLETHYLCARBAMATE

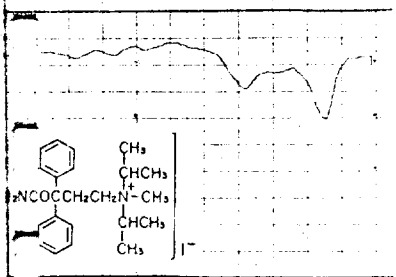

370
ISO-OCTANE

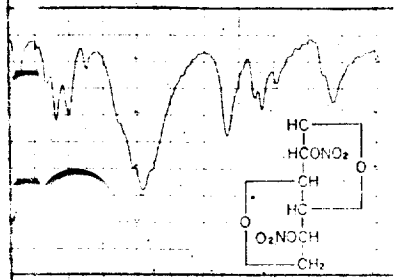

ABSORBANCE
371
IPODATE CALCIUM

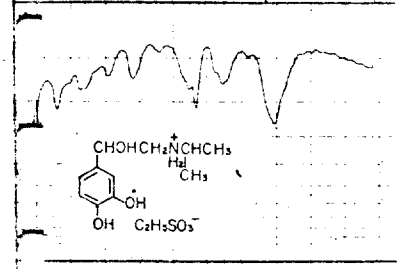

TRANSMITTANCE, %
372
IPODATE SODIUM
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
ABSORBANCE
373
ISOPREGNENONE
TRANSMITTANCE, %
374
ISOPROPAMIDE IODIDE
375
ISOSORBIDE DINITRATE
ABSORBANCE
376
ISUPREL ETHANE SULFONATE
TRANSMITTANCE, %
KANAMYCIN SULFATE
377
WAVELENGTH, μ

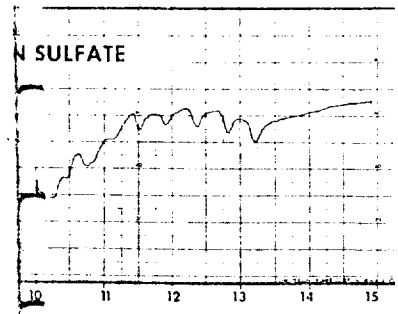


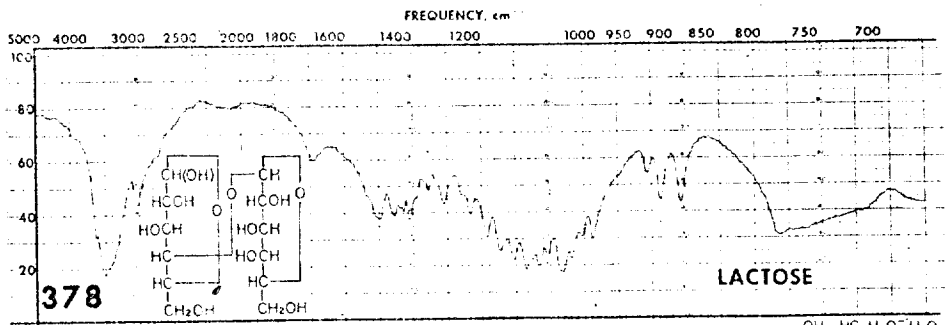


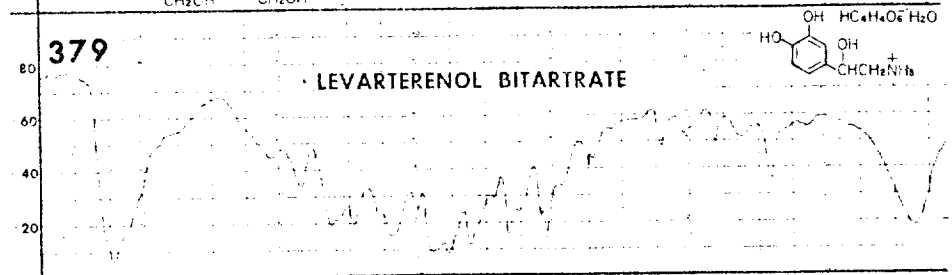


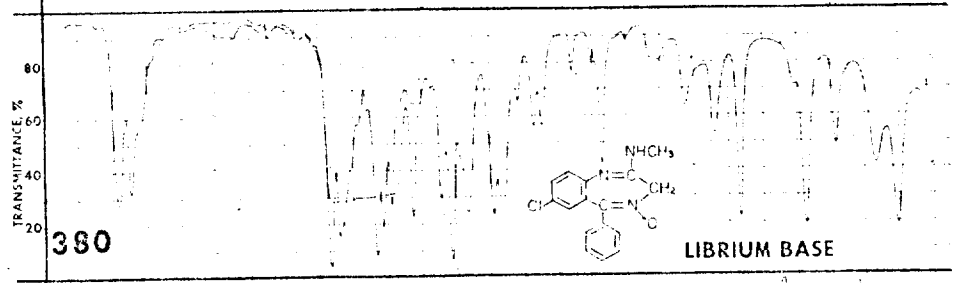


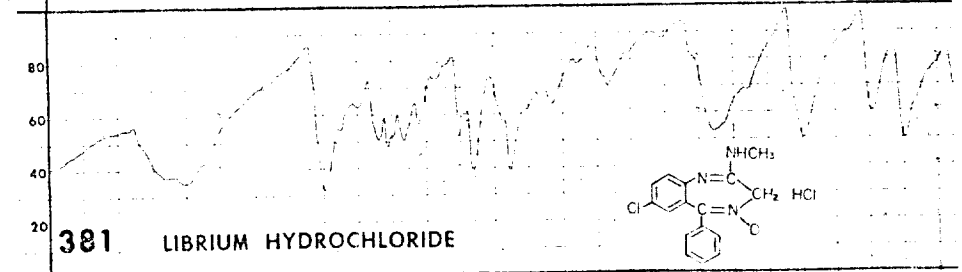
N SULFATE

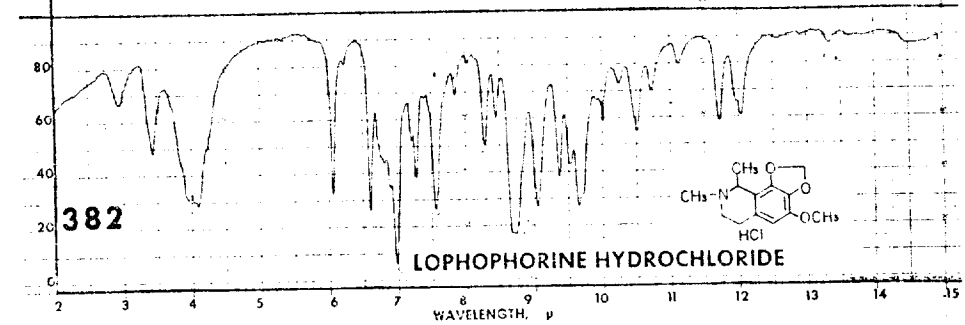


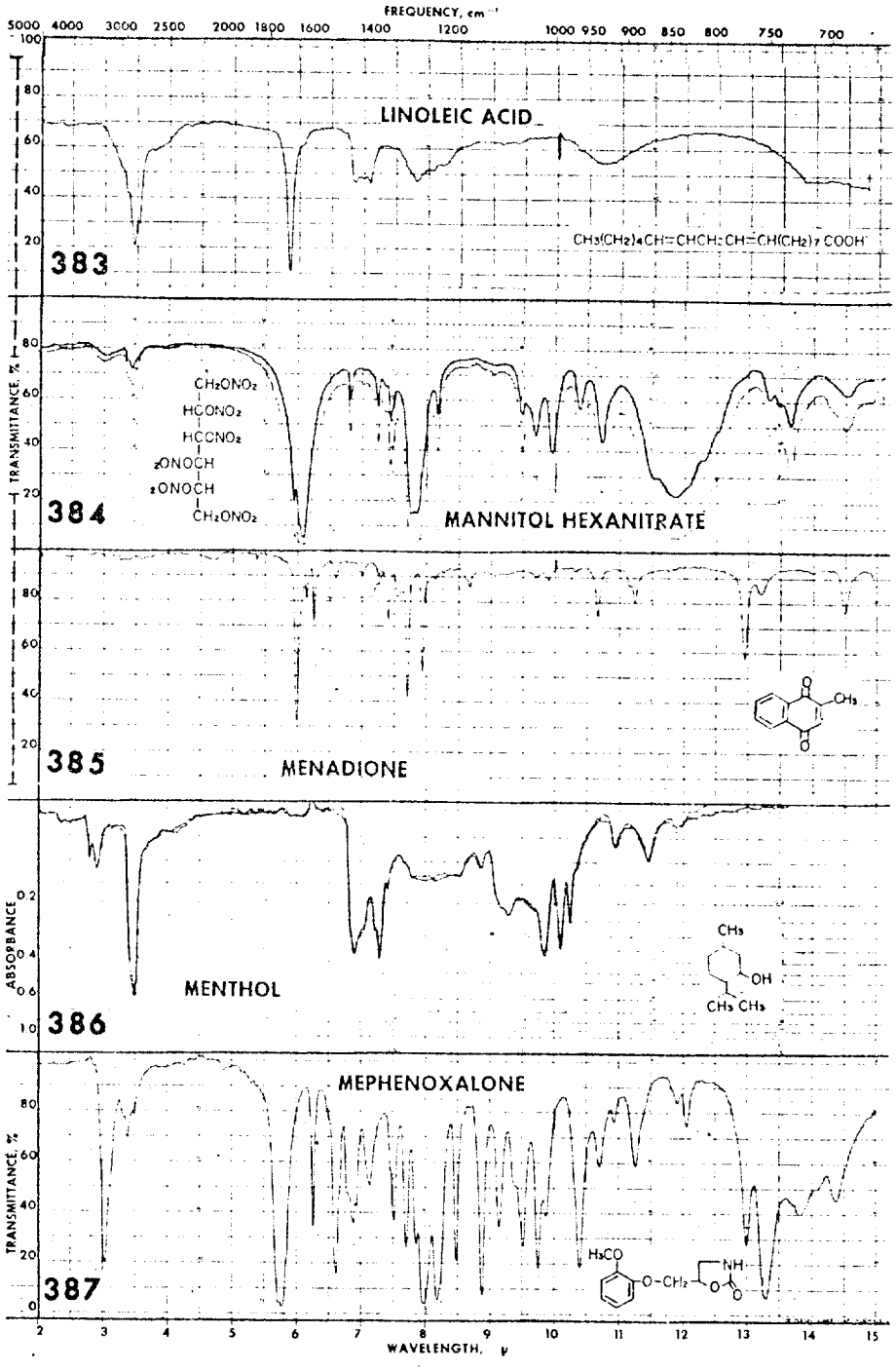

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
60
40
20
378
LACTOSE

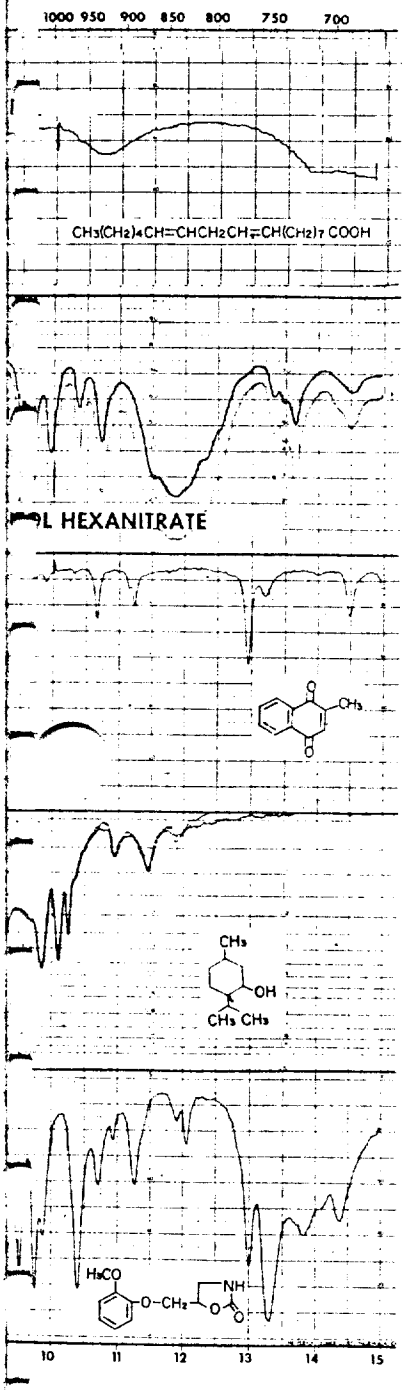

379
LEVARTERENOL BITARTRATE

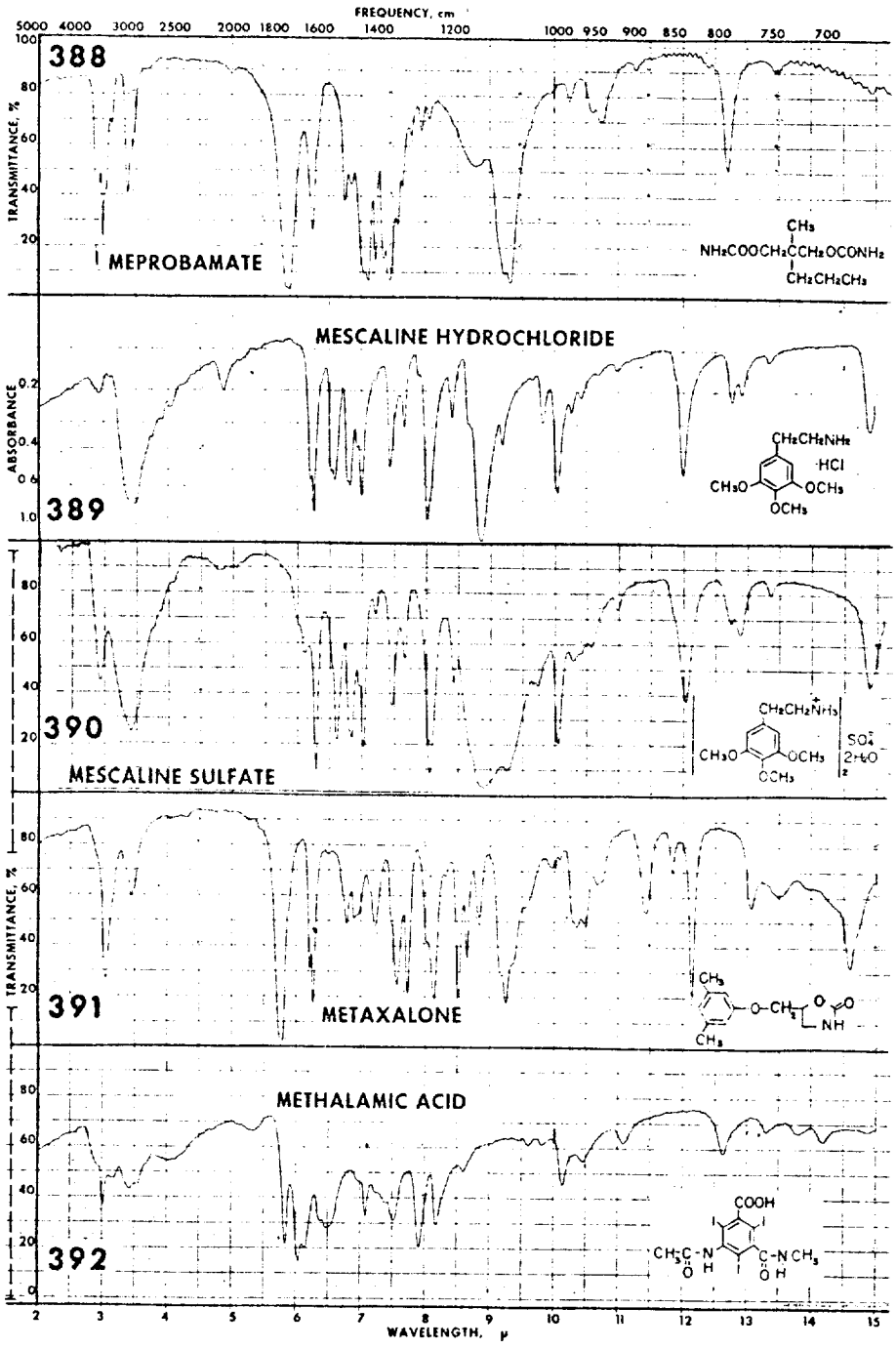

TRANSMITTANCE, %
80
60
40
20
380
LIBRIUM BASE

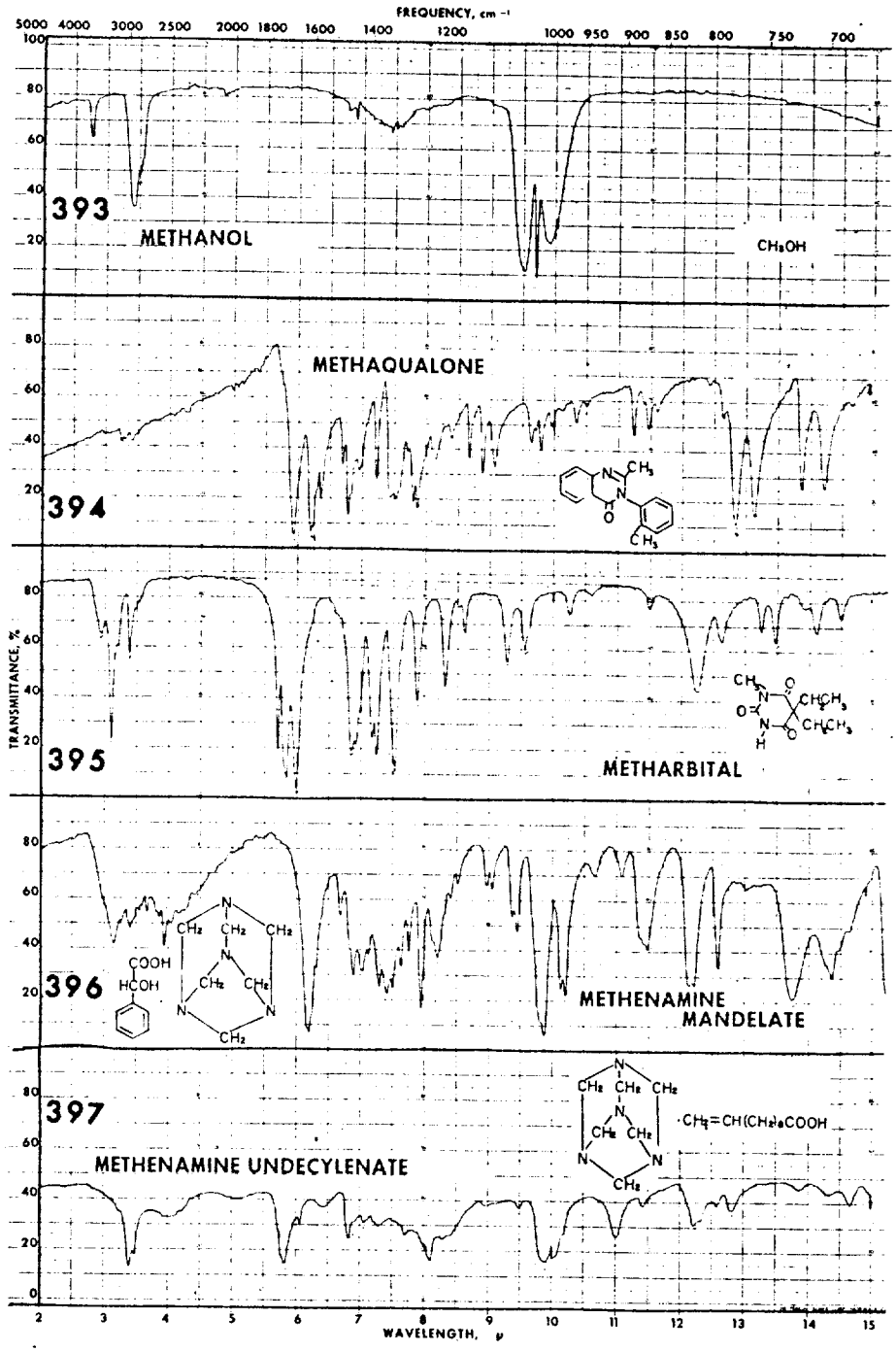

80
60
40
20
381
LIBRIUM HYDROCHLORIDE
HCl

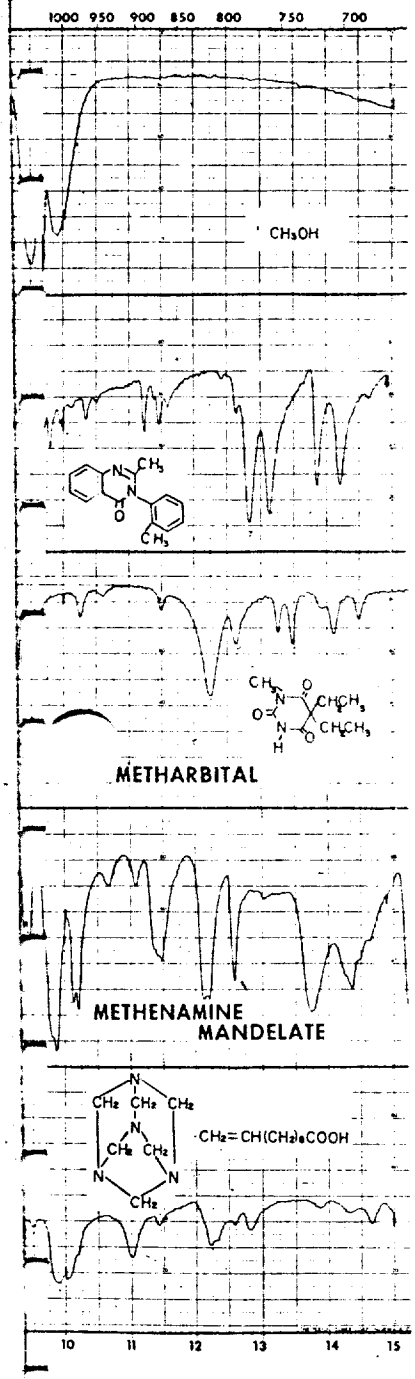

80
60
40
20
0
382
LOPHOPHORINE HYDROCHLORIDE
HCl
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

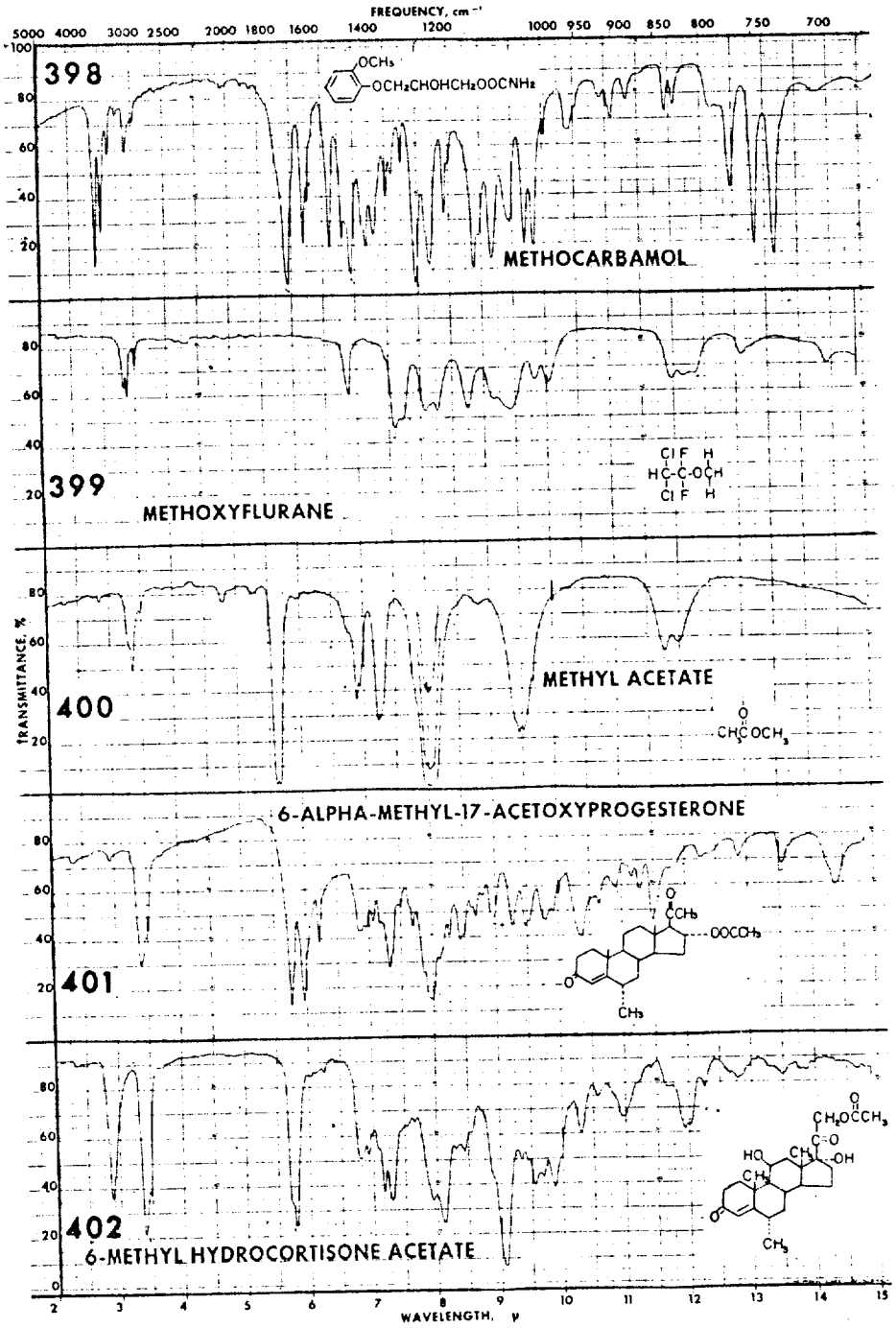

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
LINOLEIC ACID
CH3(CH2)4CH=CHCH2CH=CH(CH2)7COOH
383
TRANSMITTANCE, %
CH2ONO2
HCONO2
HCONO2
2ONOCH
2ONOCH
CH2ONO2
384
MANNITOL HEXANITRATE
385
MENADIONE
ABSORBANCE
MENTHOL
386
MEPHENOXALONE
TRANSMITTANCE, %
387
WAVELENGTH, μ

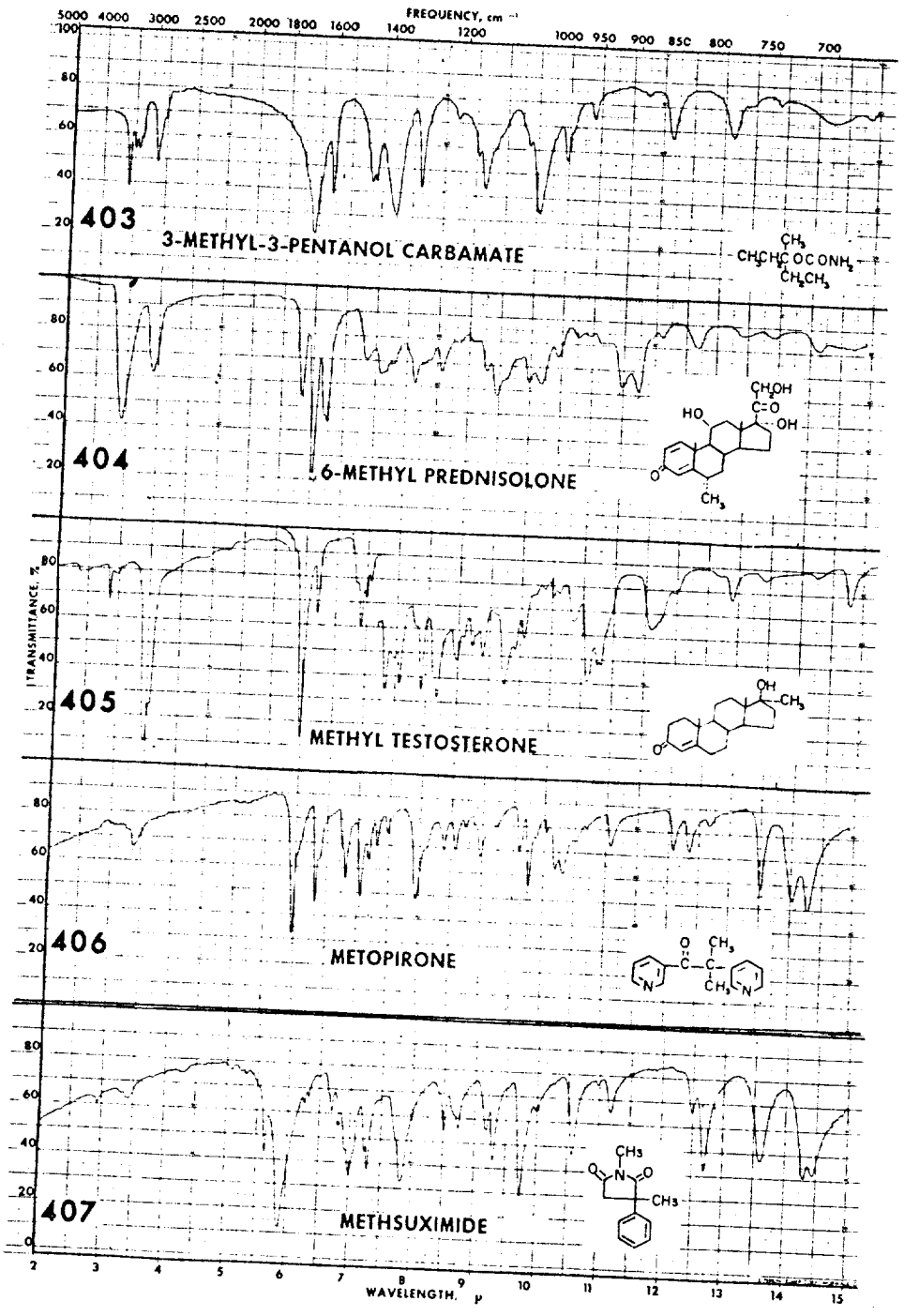

1000 950 900 850 800 750 700
CH3(CH2)4CH=CHCH2CH=CH(CH2)7 COOH
L HEXANITRATE
CH3
OH
CH3 CH3
H3CO
O-CH2
NH
10 11 12 13 14 15

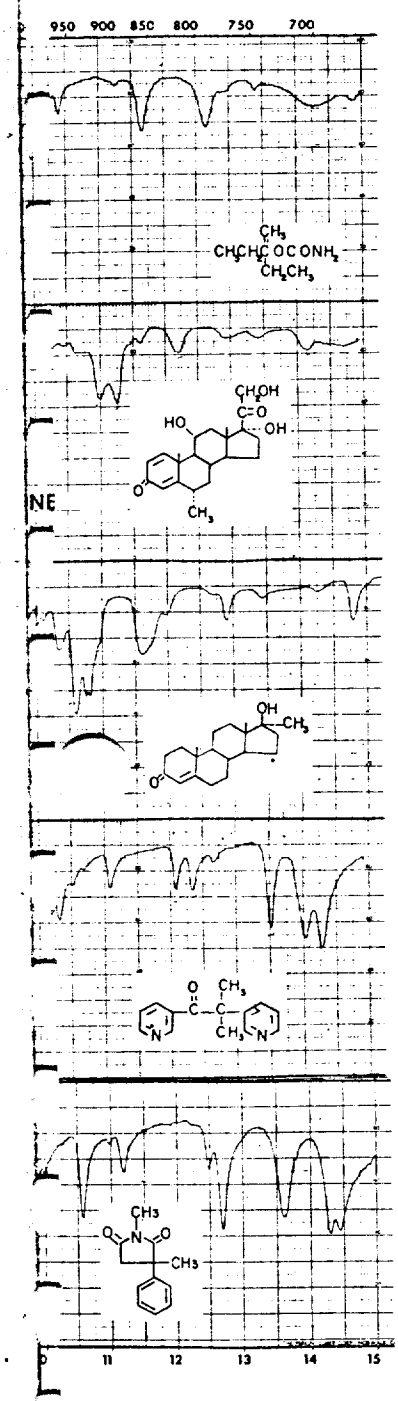

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
388
TRANSMITTANCE, %
MEPROBAMATE
NH2COOCH2CCH2OCONH2
CH2CH2CH3
MESCALINE HYDROCHLORIDE
ABSORBANCE
389
CH2CH2NH2
HCl
CH3O
OCH3
390
MESCALINE SULFATE
CH2CH2NH3
SO4
2H2O
391
METAXALONE
O-CH2
NH
METHALAMIC ACID
392
COOH
CH3C-N
C-NCH3
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

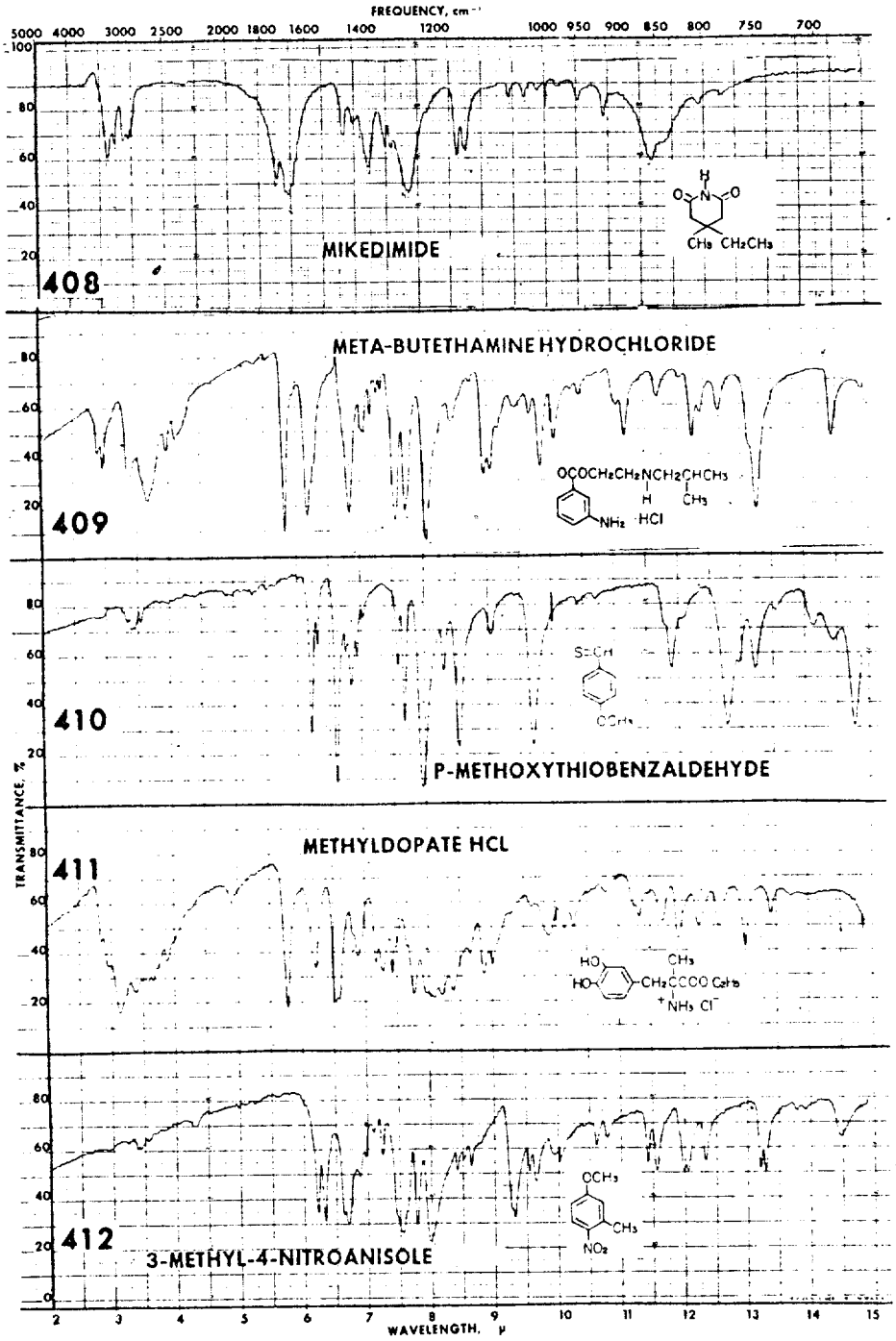

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
393
METHANOL
CH3OH
METHAQUALONE
394
395
METHARBITAL
TRANSMITTANCE, %
396
METHENAMINE MANDELATE
397
METHENAMINE UNDECYLENATE
·CH2=CH(CH2)8COOH
WAVELENGTH, μ

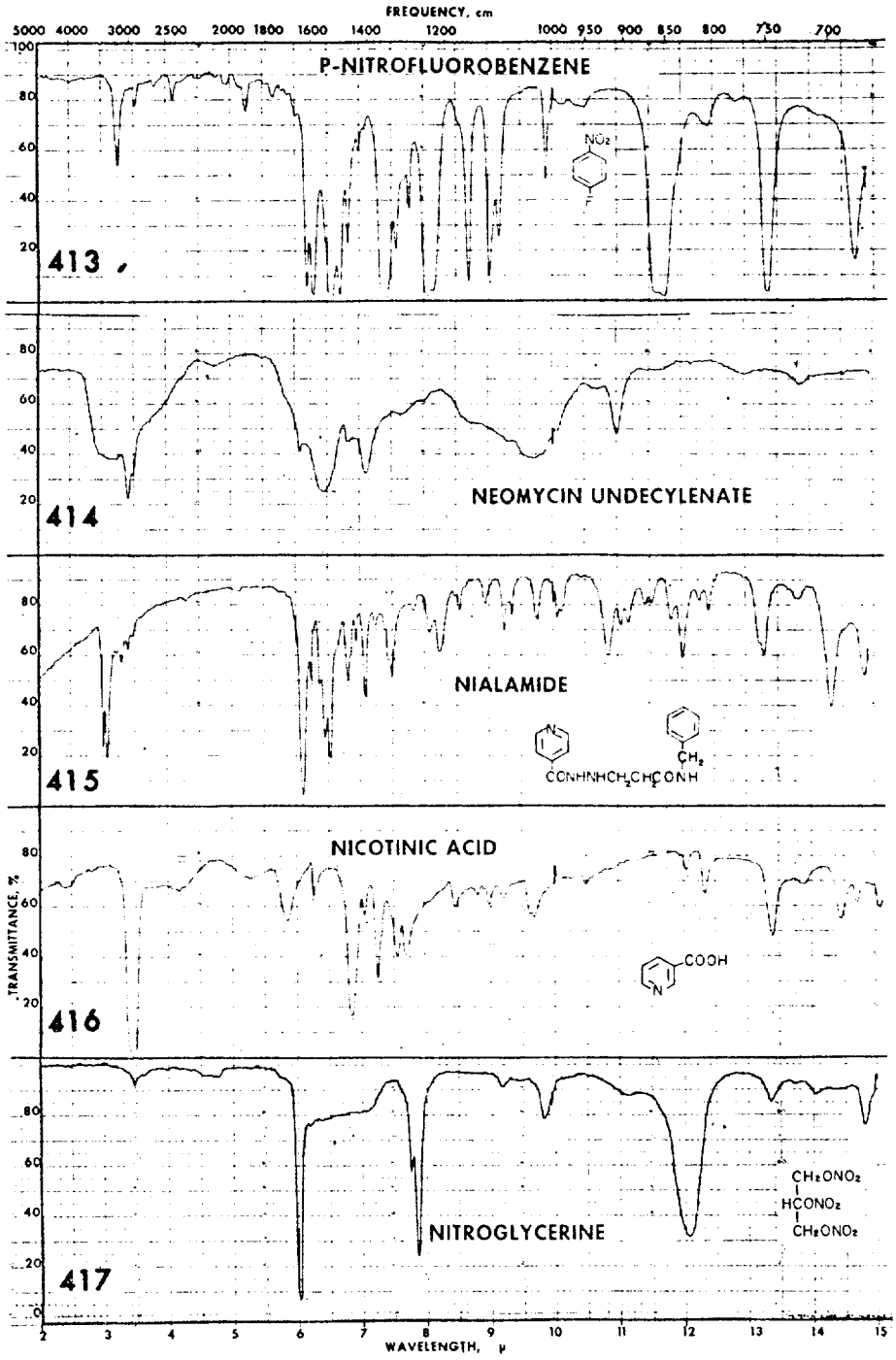

1000 950 900 850 800 750 700
CH3OH
METHARBITAL
METHENAMINE MANDELATE
·CH2=CH(CH2)8COOH
10 11 12 13 14 15

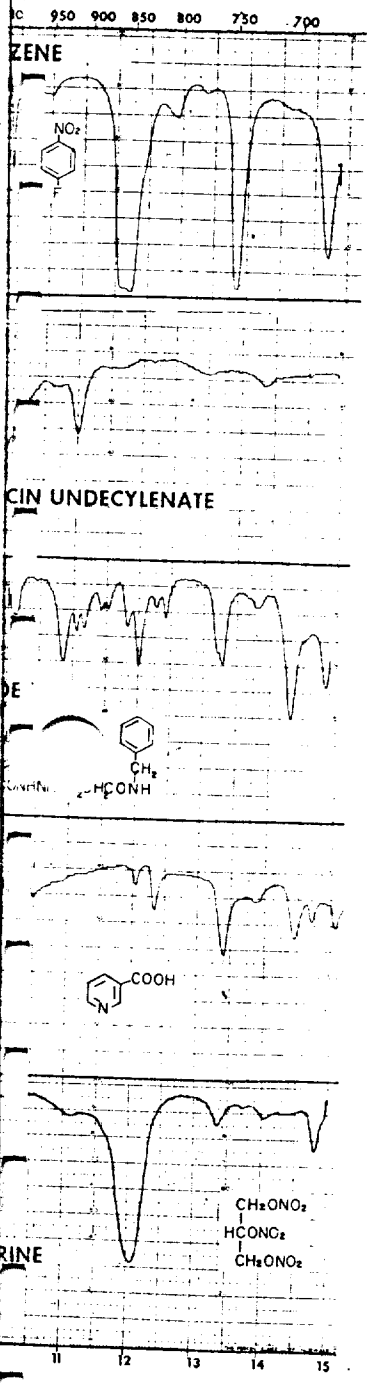

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
398
METHOCARBAMOL
399
METHOXYFLURANE
400
METHYL ACETATE
6-ALPHA-METHYL-17-ACETOXYPROGESTERONE
401
402
6-METHYL HYDROCORTISONE ACETATE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

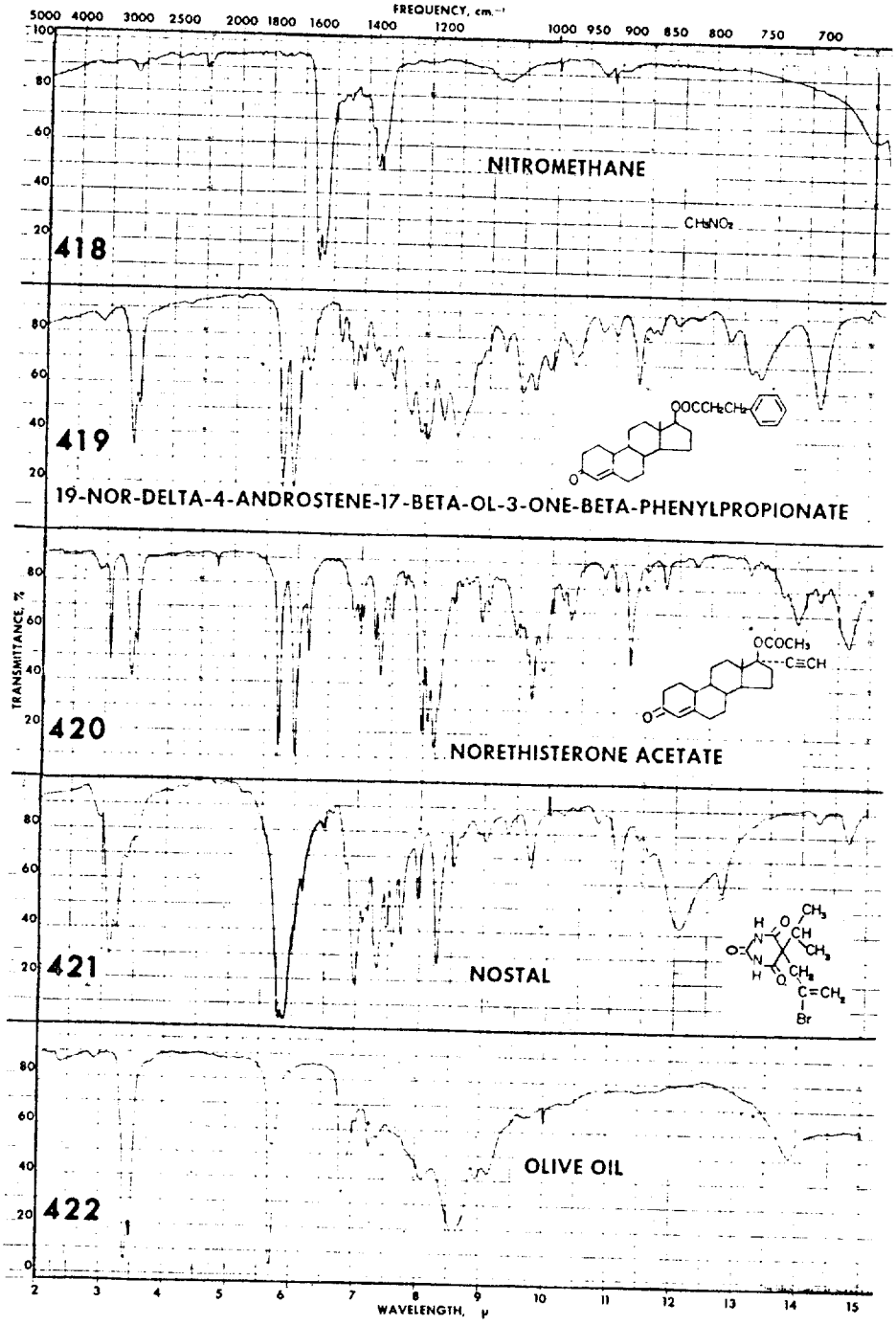

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
403
3-METHYL-3-PENTANOL CARBAMATE
404
6-METHYL PREDNISOLONE
405
METHYL TESTOSTERONE
406
METOPIRONE
407
METHSUXIMIDE
TRANSMITTANCE %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

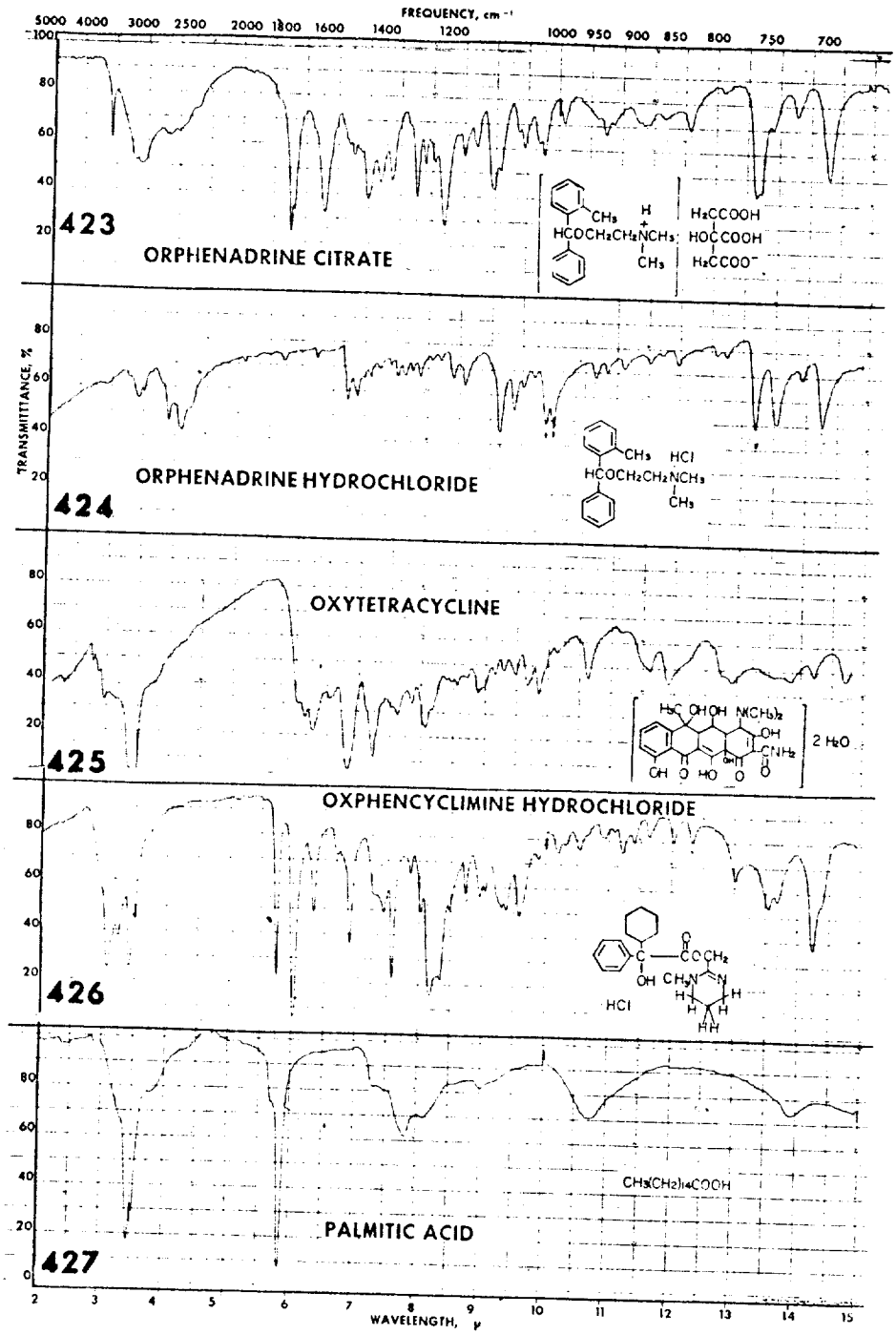

950 900 850 800 750 700
NE
0 11 12 13 14 15

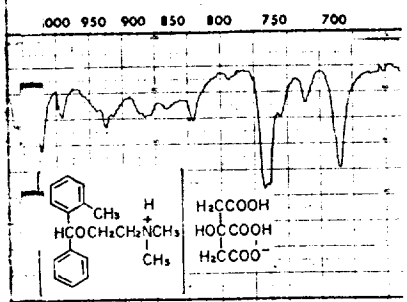

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
408
MIKEDIMIDE
409
META-BUTETHAMINE HYDROCHLORIDE
410
P-METHOXYTHIOBENZALDEHYDE
411
METHYLDOPATE HCL
412
3-METHYL-4-NITROANISOLE
TRANSMITTANCE, %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

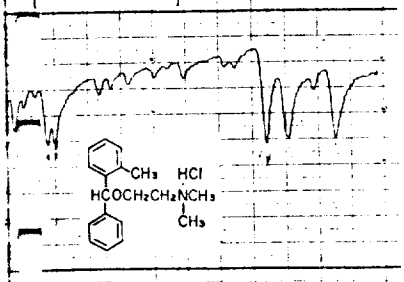

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
P-NITROFLUOROBENZENE
413
NEOMYCIN UNDECYLENATE
414
NIALAMIDE
415
NICOTINIC ACID
416
NITROGLYCERINE
417
TRANSMITTANCE, %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

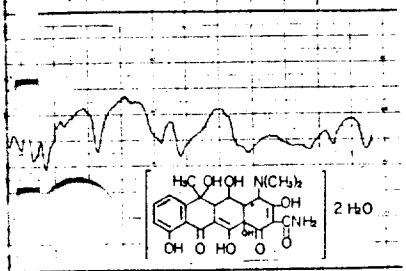

950 900 850 800 750 700
ZENE
NO2
CIN UNDECYLENATE
DE
CH2
HCONH
COOH
N
CH2ONO2
HCONO2
CH2ONO2
RINE
11 12 13 14 15


FREQUENCY, cm.-1
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
NITROMETHANE
CH3NO2
418
419
OOCCH2CH2
19-NOR-DELTA-4-ANDROSTENE-17-BETA-OL-3-ONE-BETA-PHENYLPROPIONATE
TRANSMITTANCE, %
420
OCOCH3
C≡CH
NORETHISTERONE ACETATE
421
NOSTAL
CH3
CH
C=CH2
Br
422
OLIVE OIL
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

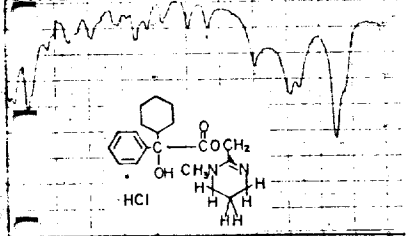

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
423
ORPHENADRINE CITRATE
424
ORPHENADRINE HYDROCHLORIDE
425
OXYTETRACYCLINE
426
OXPHENCYCLIMINE HYDROCHLORIDE
427
PALMITIC ACID
CH3(CH2)14COOH
2 H2O
HCl
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

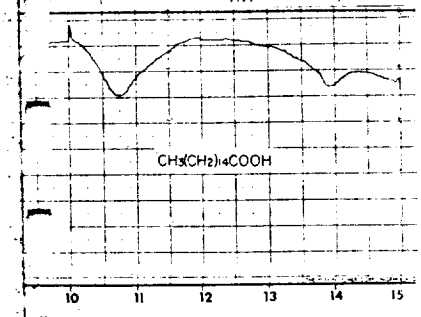


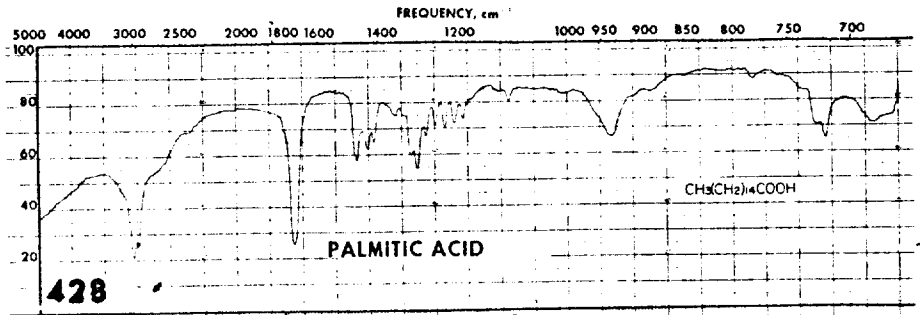


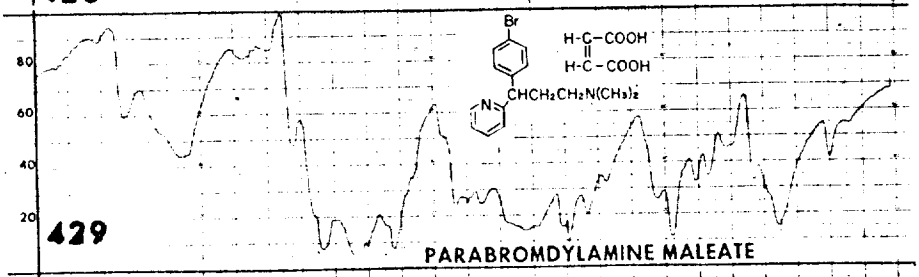


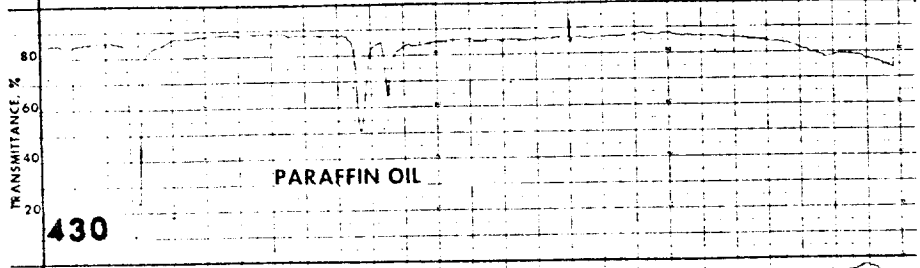
E HYDROCHLORIDE

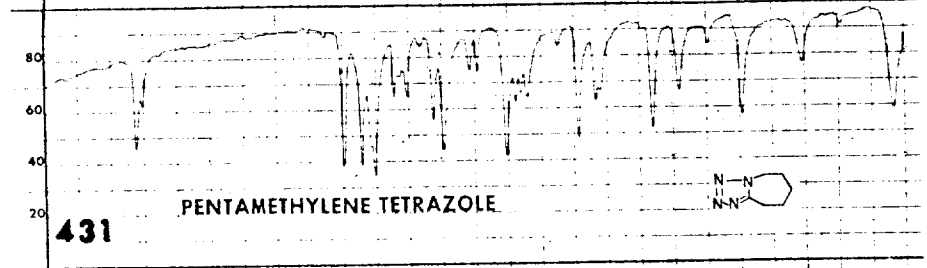


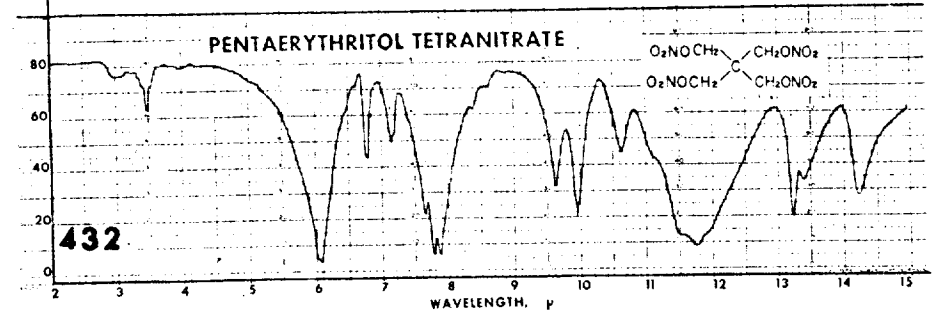


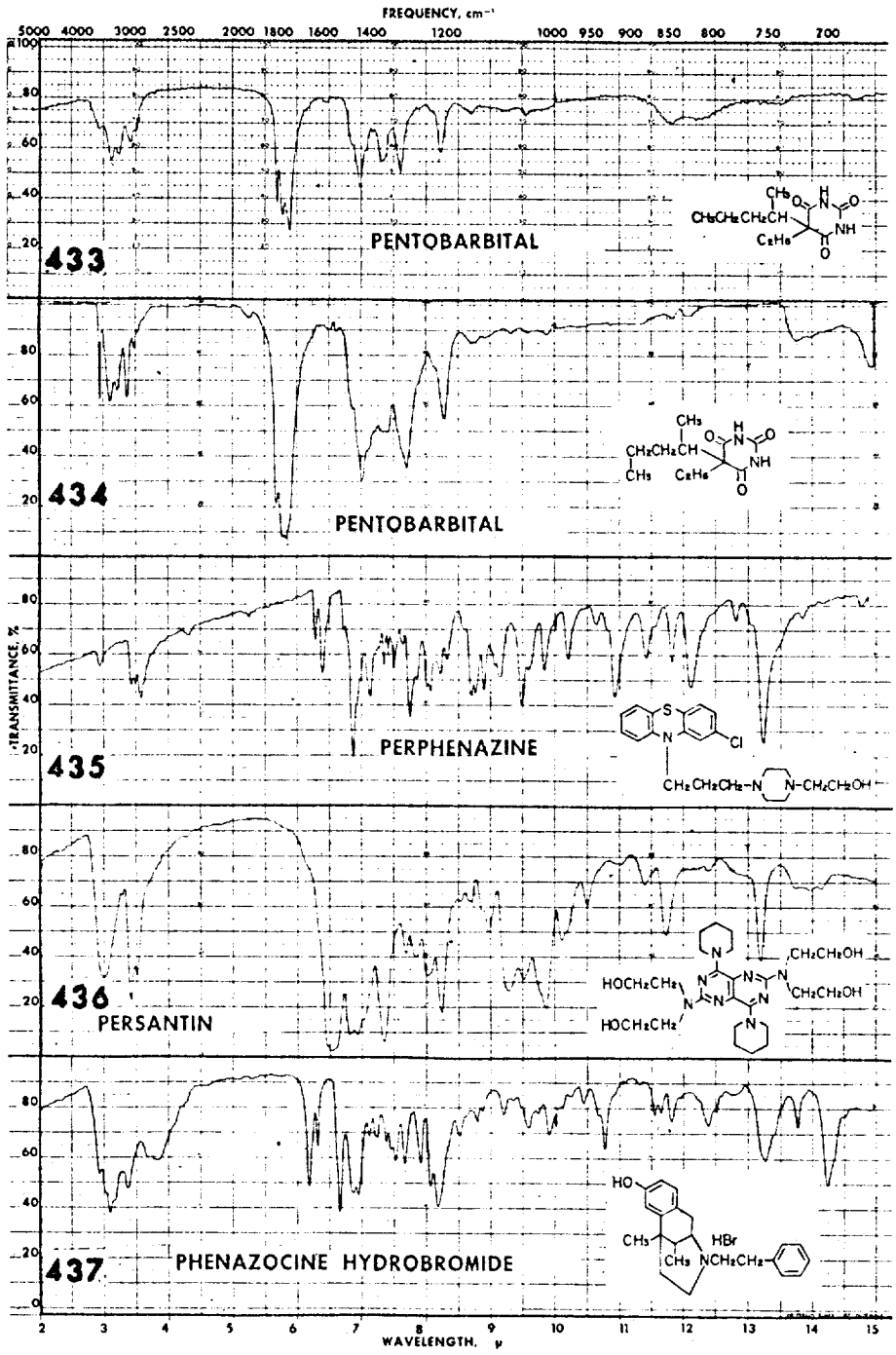


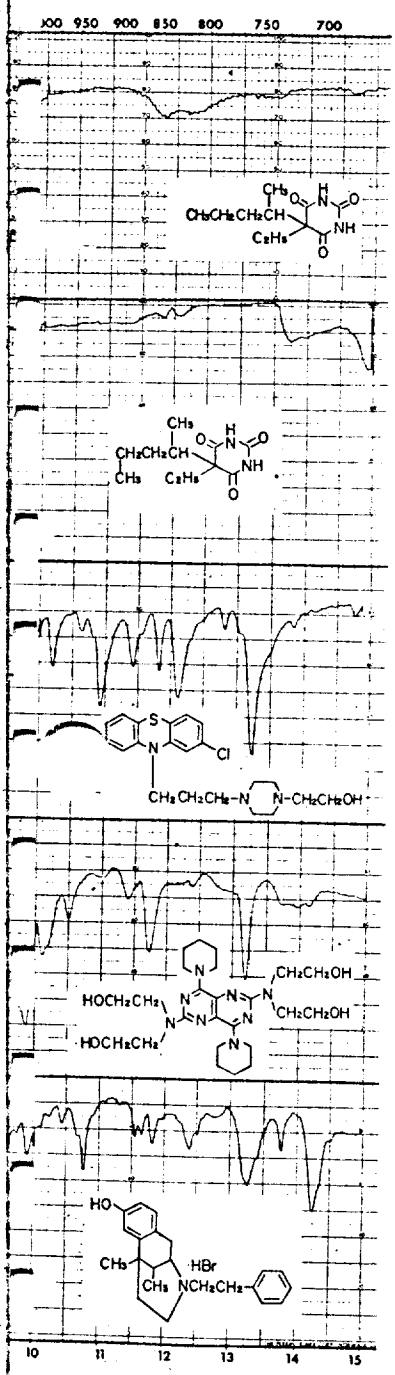


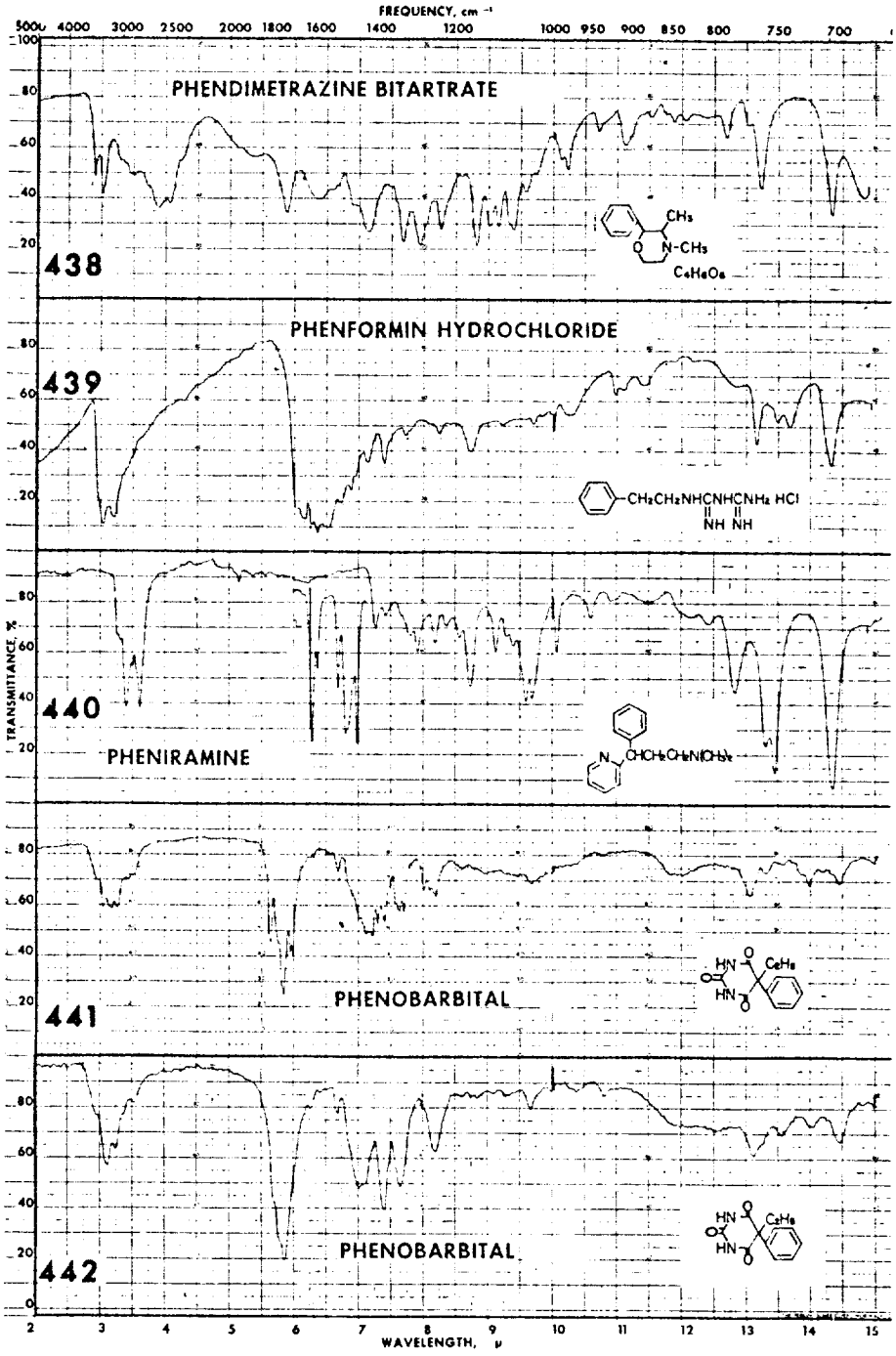


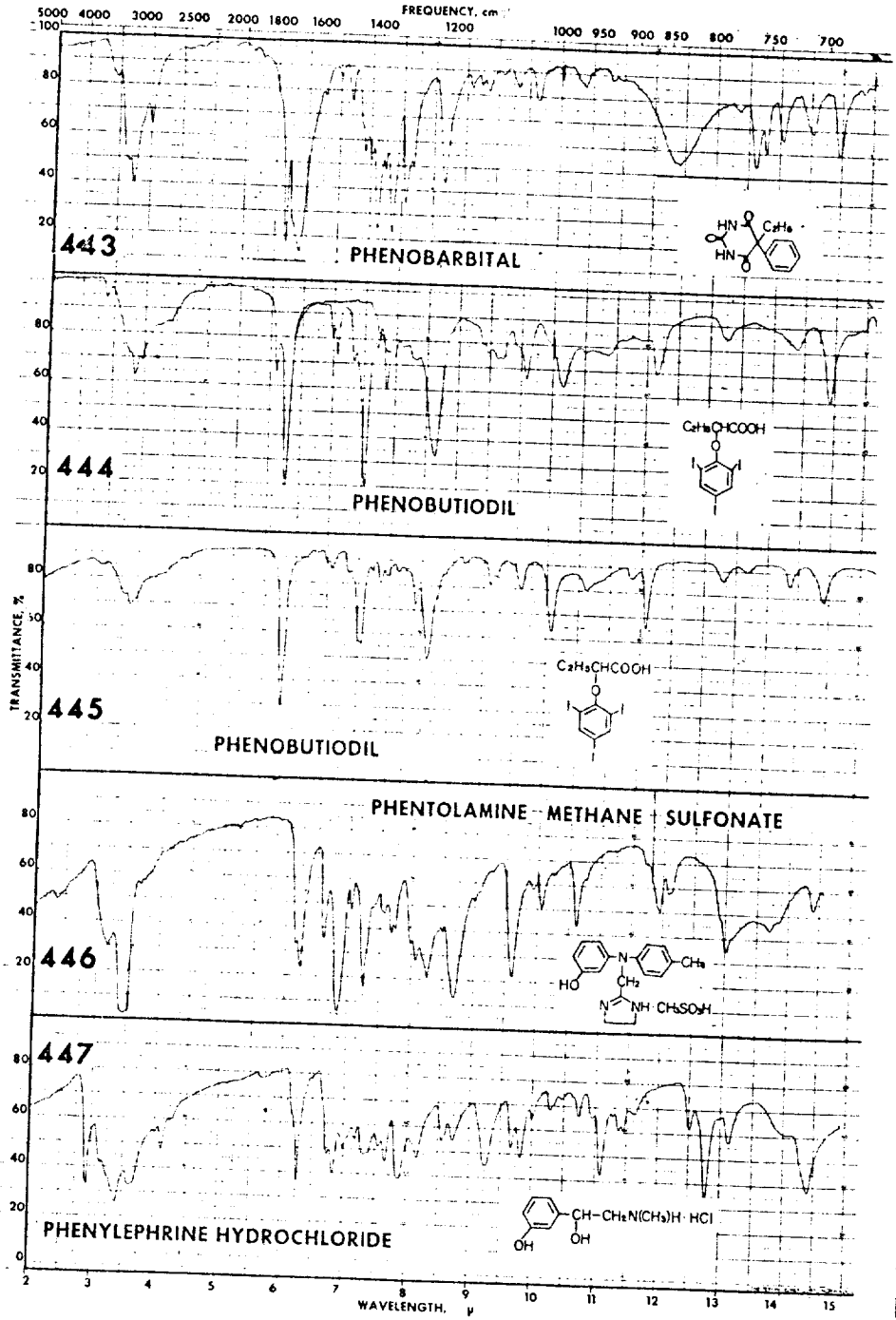


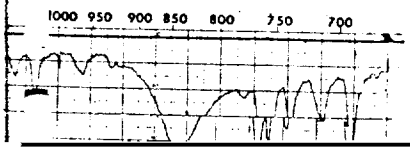


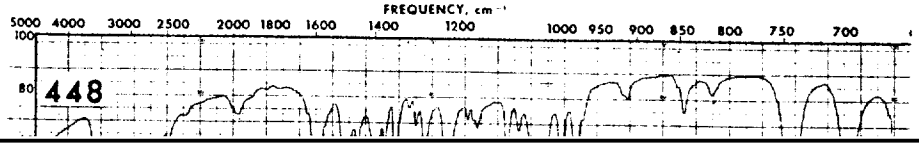

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
433
PENTOBARBITAL
434
PENTOBARBITAL
435
PERPHENAZINE
436
PERSANTIN
437
PHENAZOCINE HYDROBROMIDE
TRANSMITTANCE, %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

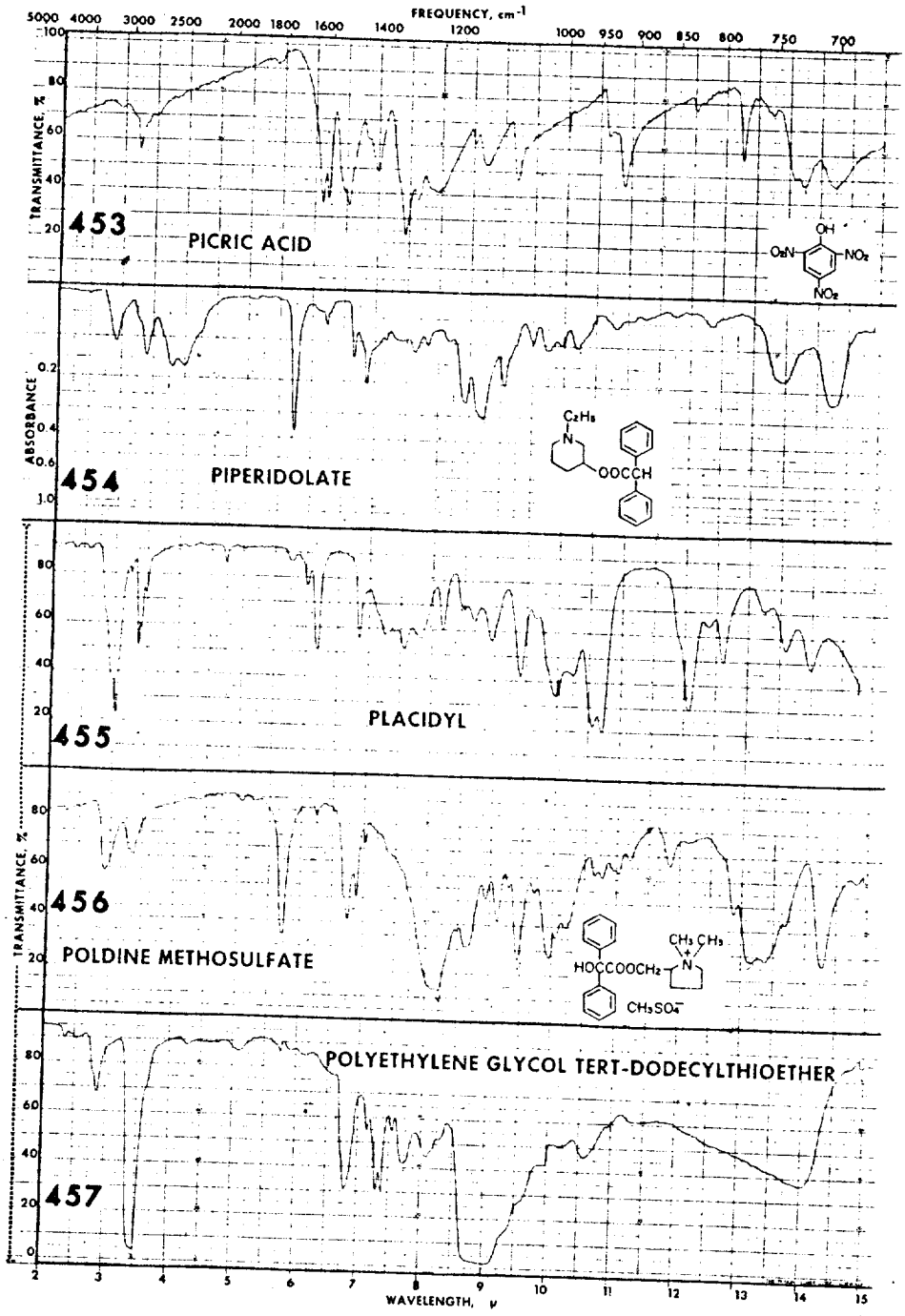

100 950 900 850 800 750 700
10 11 12 13 14 15

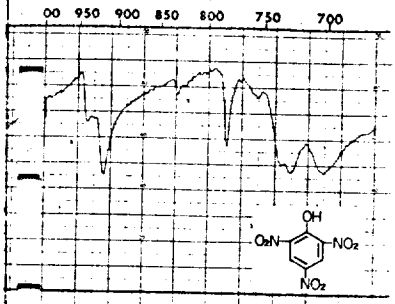

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
PHENDIMETRAZINE BITARTRATE
438
PHENFORMIN HYDROCHLORIDE
439
TRANSMITTANCE %
440
PHENIRAMINE
441
PHENOBARBITAL
442
PHENOBARBITAL
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

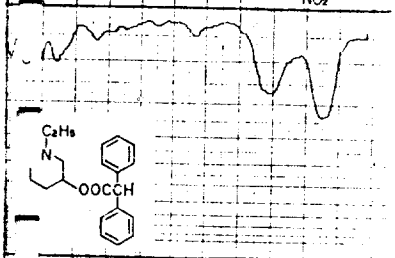

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
443
PHENOBARBITAL
444
PHENOBUTIODIL
445
PHENOBUTIODIL
PHENTOLAMINE METHANE SULFONATE
446
447
PHENYLEPHRINE HYDROCHLORIDE
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

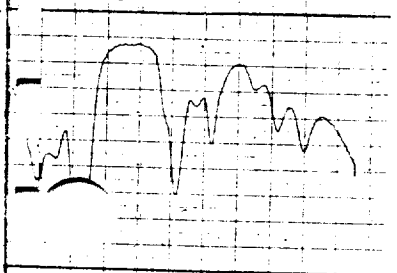

1000 950 900 850 800 750 700

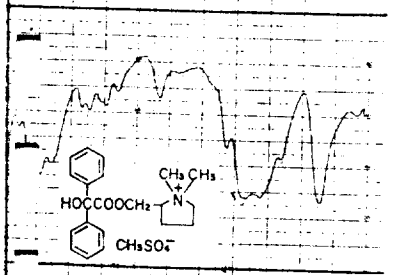

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
448

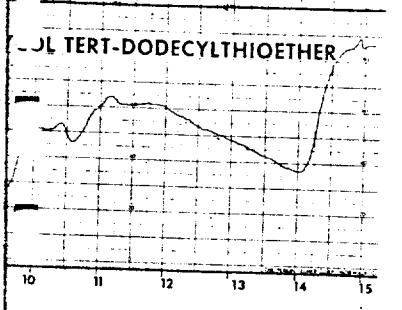

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
453
PICRIC ACID
ABSORBANCE
454
PIPERIDOLATE
455
PLACIDYL
TRANSMITTANCE, %
456
POLDINE METHOSULFATE
457
POLYETHYLENE GLYCOL TERT-DODECYLTHIOETHER
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

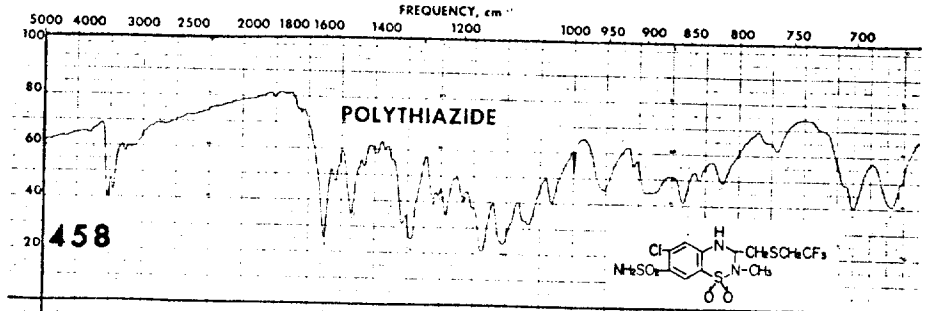

00 950 900 850 800 750 700
OH
O2N
NO2
NO2

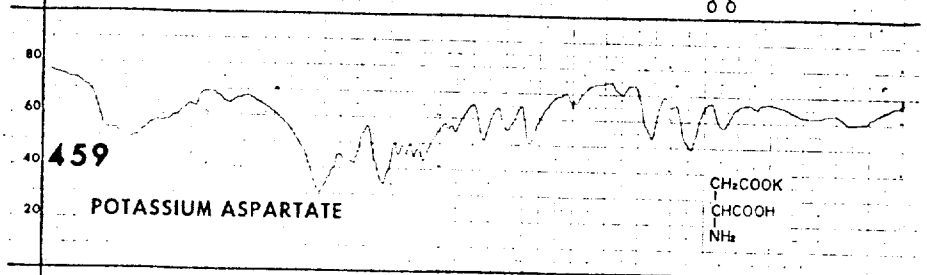

C2H5
N
OOCCH

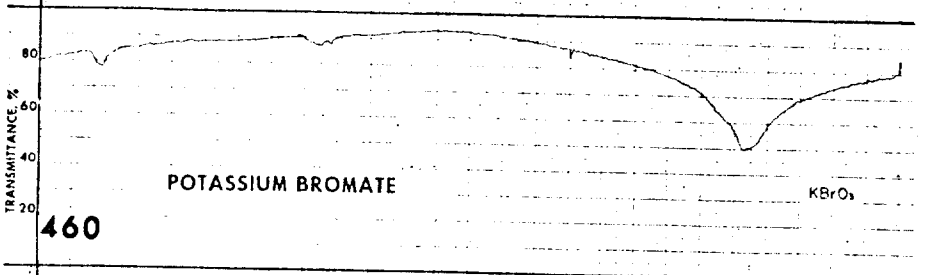


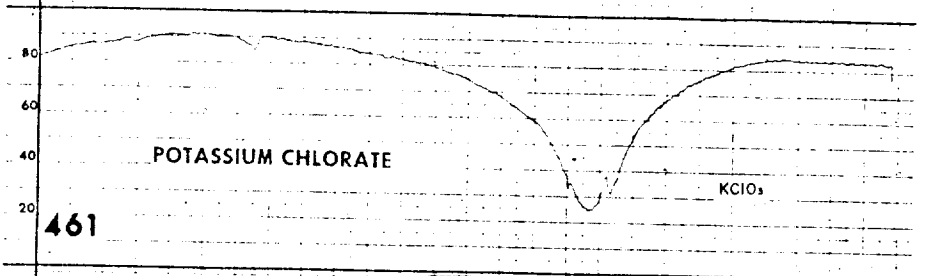

CH3 CH3
HOCCOOCH2
N
CH3SO4

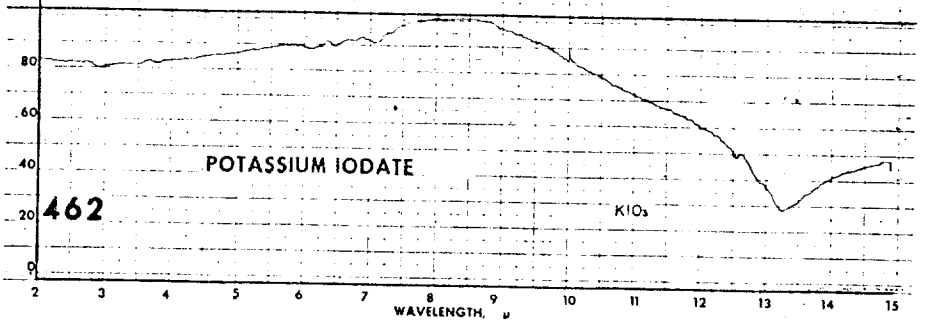

-OL TERT-DODECYLTHIOETHER
10
11
12
13
14
15

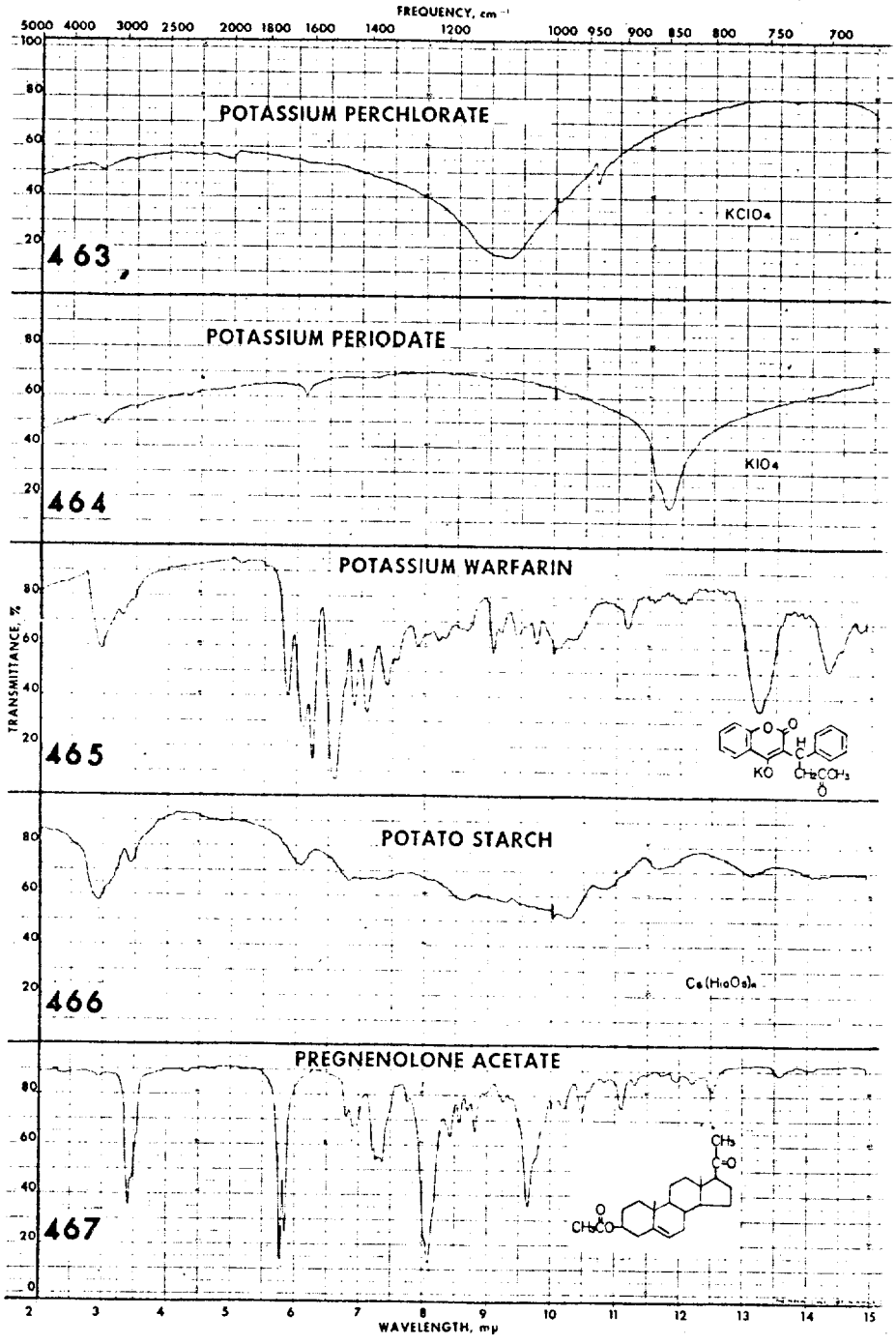

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
100
80
60
40
20
458
POLYTHIAZIDE
Cl
NH2SO2
H
N
CH2SCH2CF3
N-CH3
S
O O

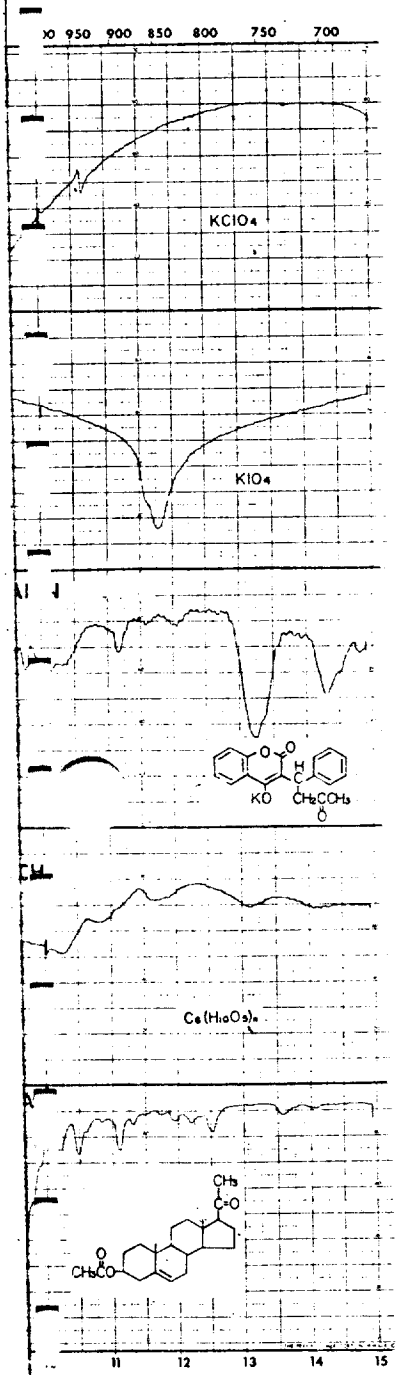

80
60
40
20
459
POTASSIUM ASPARTATE
CH2COOK
CHCOOH
NH2

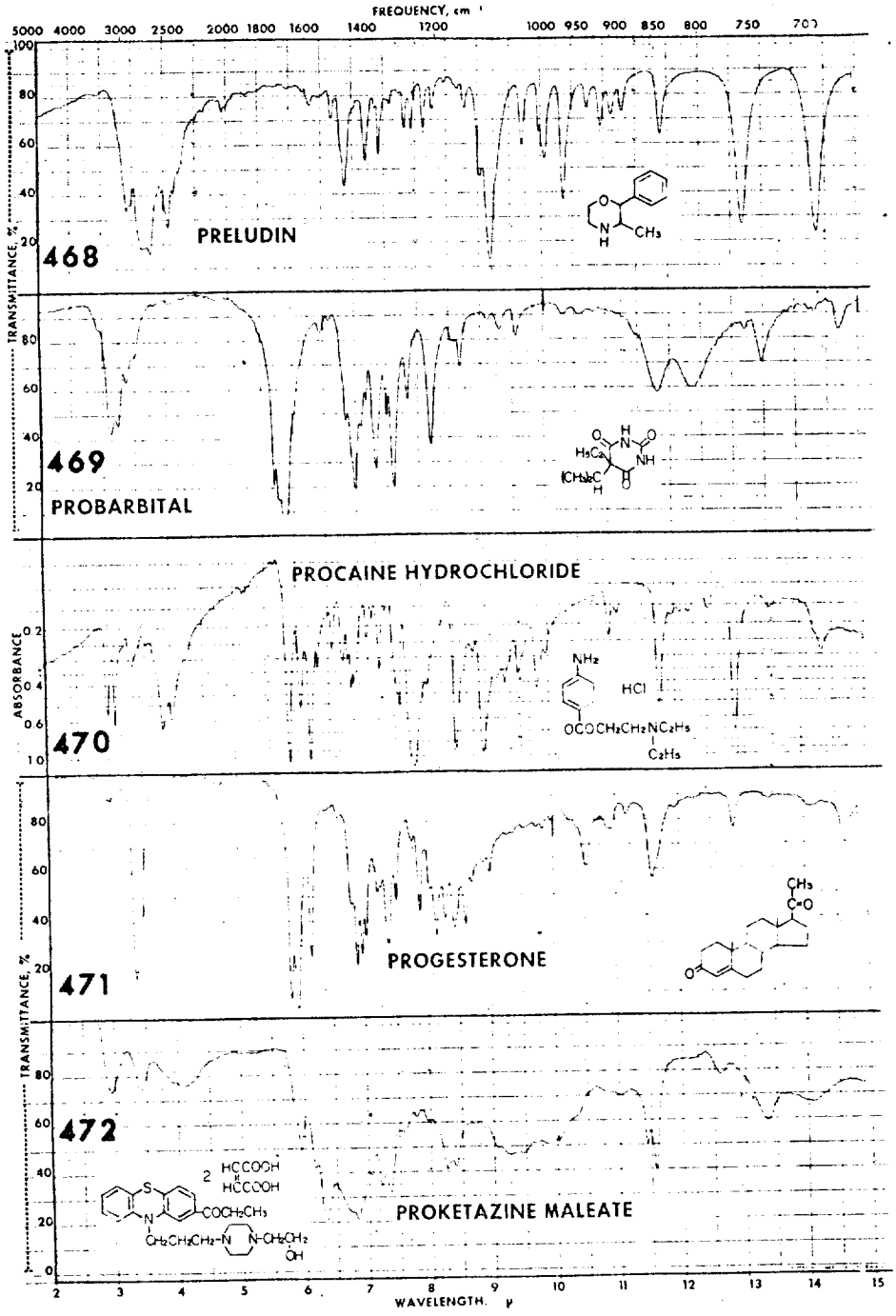

TRANSMITTANCE, %
80
60
40
20
POTASSIUM BROMATE
KBrO3
460

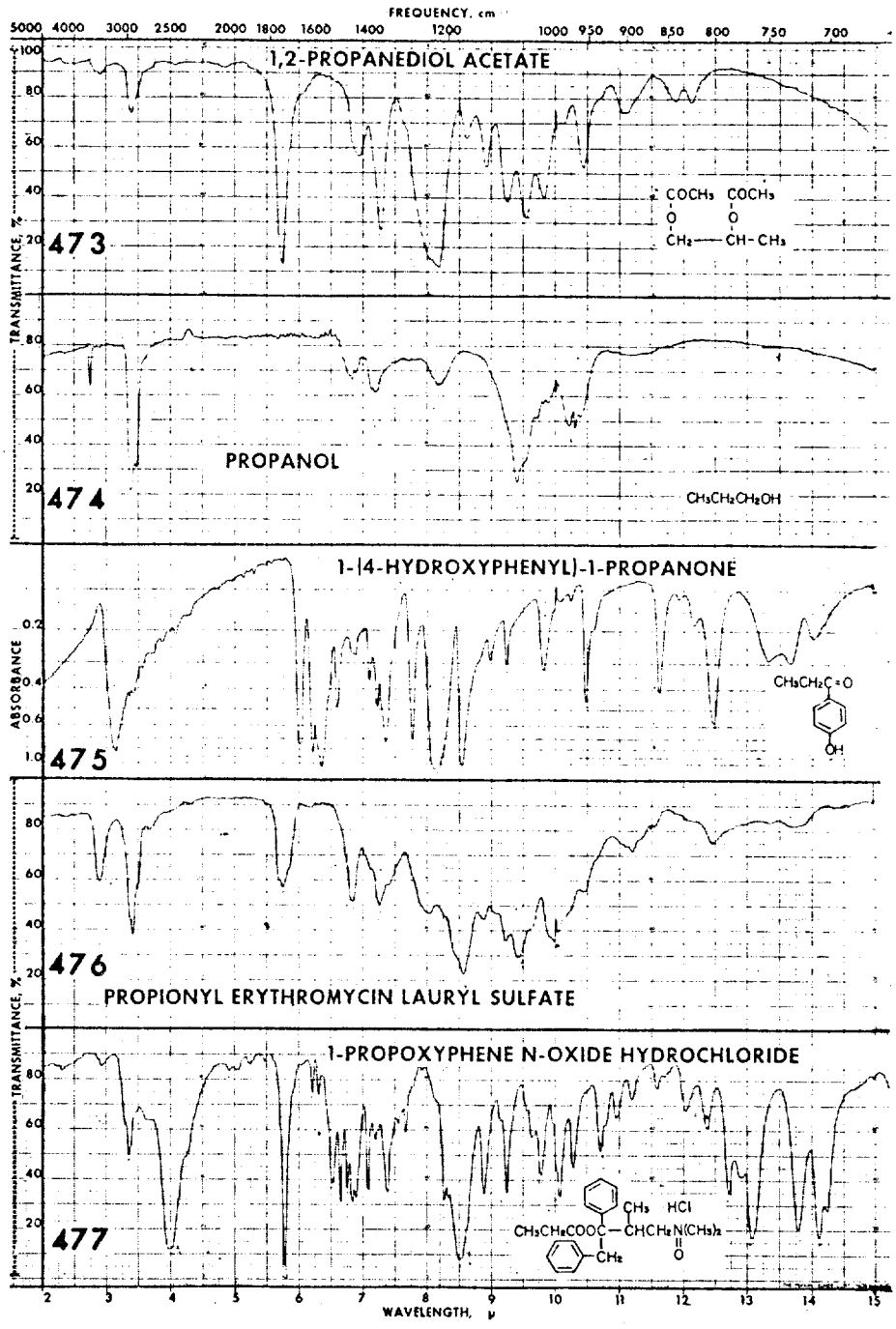

80
60
40
20
POTASSIUM CHLORATE
KClO3
461

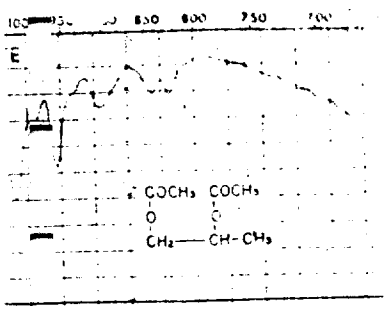

80
60
40
20
0
POTASSIUM IODATE
KIO3
462
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH, μ

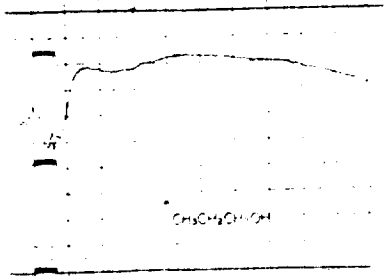

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
POTASSIUM PERCHLORATE
KClO4
463
POTASSIUM PERIODATE
KIO4
464
POTASSIUM WARFARIN
TRANSMITTANCE, %
465
POTATO STARCH
(C6H10O5)n
466
PREGNENOLONE ACETATE
467
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, mμ


950 900 850 800 750 700
$KClO_4$
KIO_4
KO
CH_2COCH_3
O
$C_6(H_{10}O_5)_n$
CH_3
C=O
CH_3CO
11 12 13 14 15

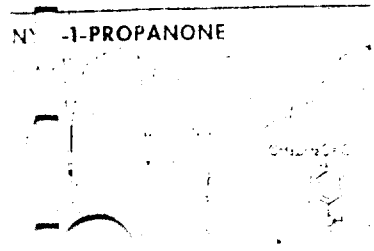

FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
100
80
60
40
20
468
PRELUDIN
CH_3
469
PROBARBITAL
H_5C_2
$(CH_3)_2CH$
NH
ABSORBANCE
0.2
0.4
0.6
1.0
PROCAINE HYDROCHLORIDE
NH_2
HCl
$OCOCH_2CH_2NC_2H_5$
C_2H_5
470
471
PROGESTERONE
CH_3
C=O
472
2 HCCOOH
HCCOOH
$COCH_2CH_3$
$CH_2CH_2CH_2-N$
$N-CH_2CH_2$
OH
PROKETAZINE MALEATE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

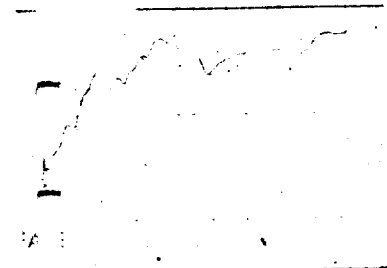

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
1,2-PROPANEDIOL ACETATE
473
TRANSMITTANCE, %
PROPANOL
474
CH3CH2CH2OH
1-(4-HYDROXYPHENYL)-1-PROPANONE
ABSORBANCE
475
476
PROPIONYL ERYTHROMYCIN LAURYL SULFATE
1-PROPOXYPHENE N-OXIDE HYDROCHLORIDE
477
WAVELENGTH, μ



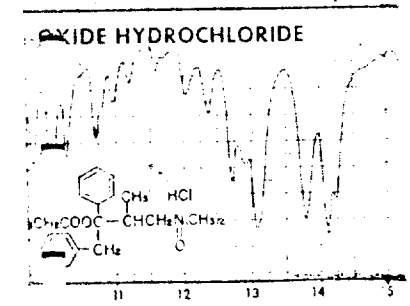


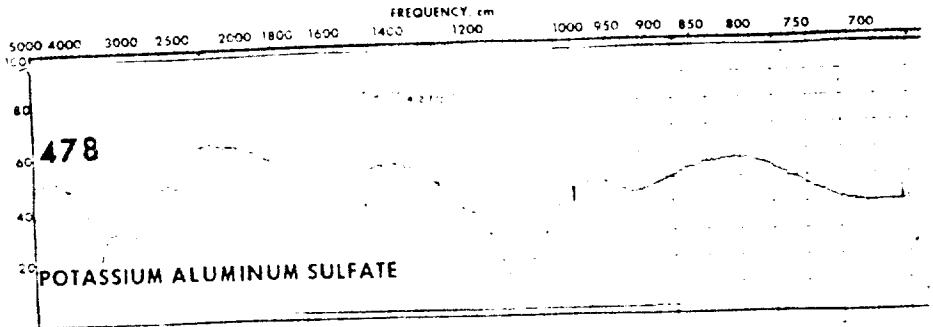
N -1-PROPANONE

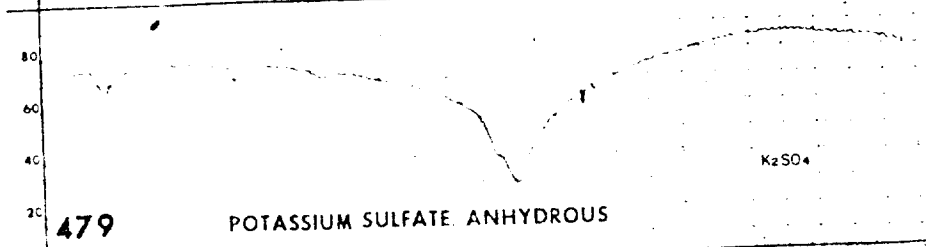


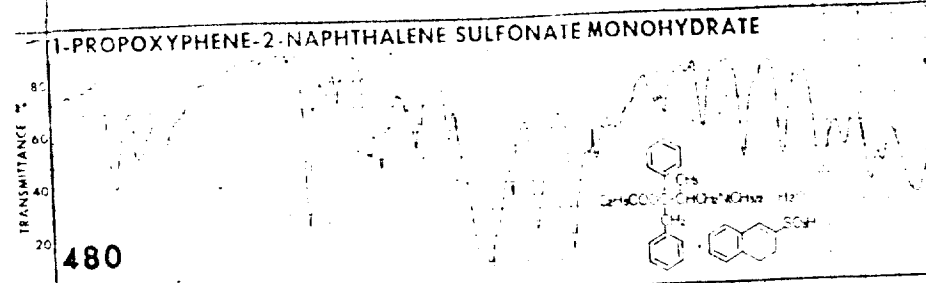


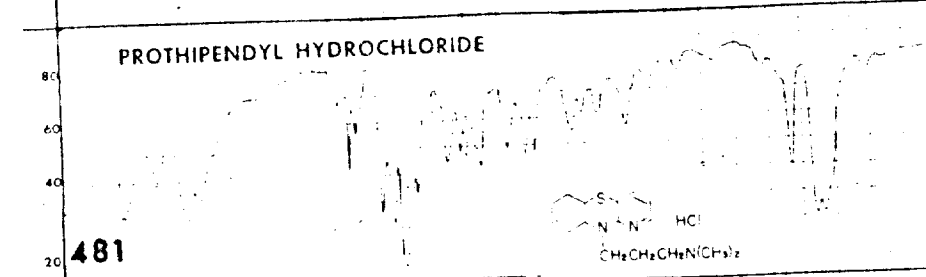
...XIDE HYDROCHLORIDE

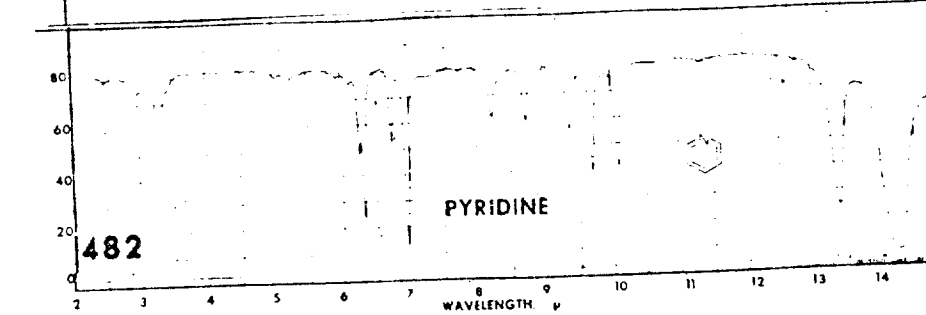


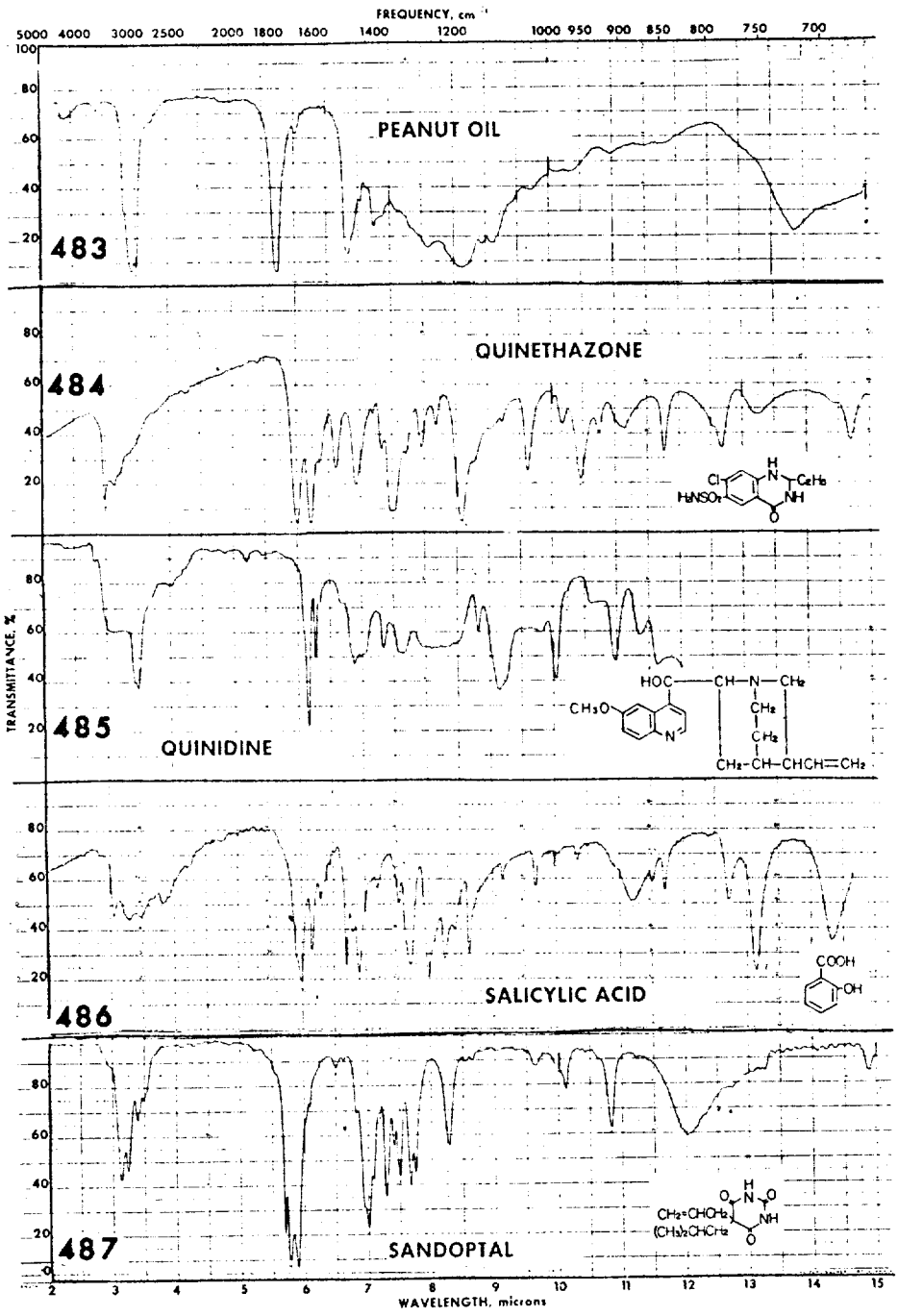


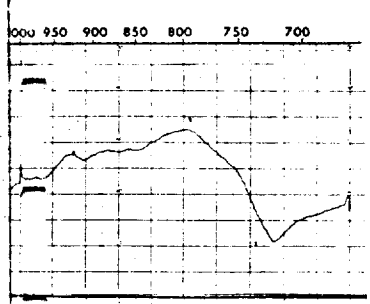




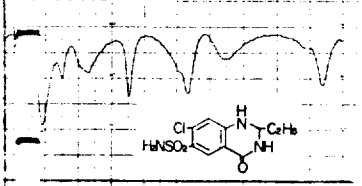


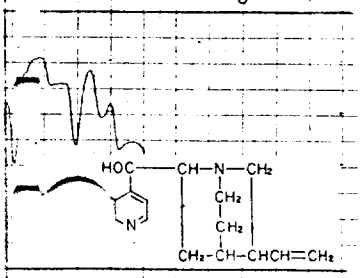



FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
483
PEANUT OIL
484
QUINETHAZONE
485
QUINIDINE
486
SALICYLIC ACID
487
SANDOPTAL
WAVELENGTH, microns
2 3 4 5 6 7 8 9 10 11 12 13 14 15

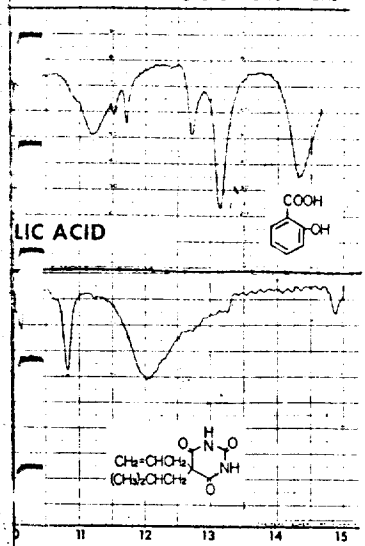


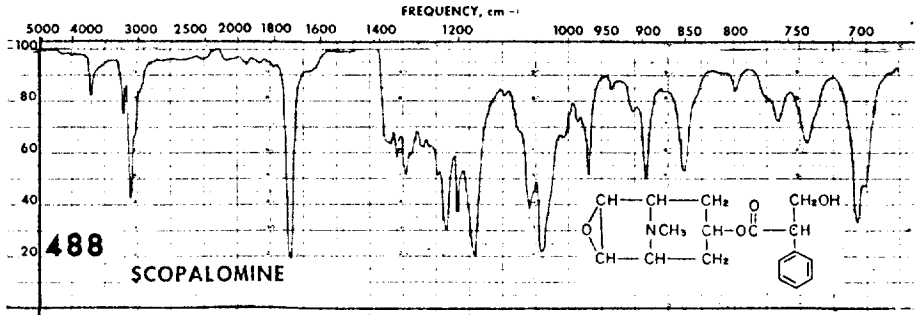
THAZONE

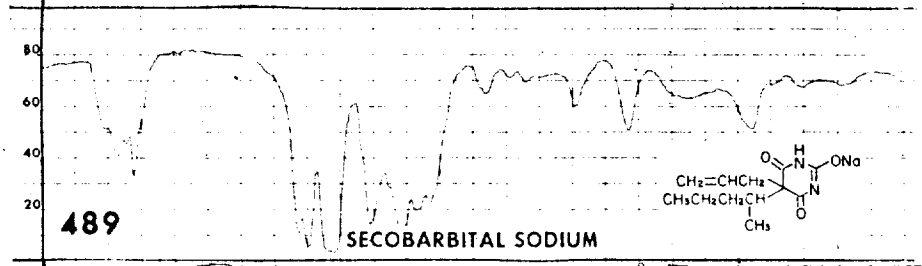


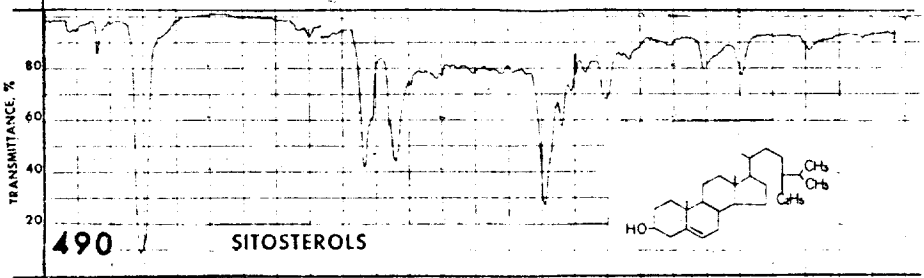


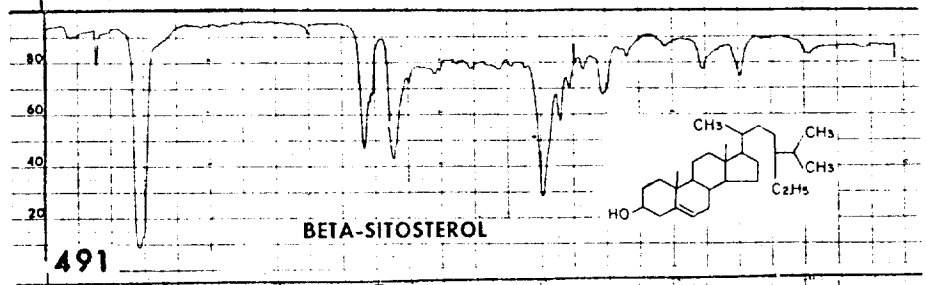
LIC ACID

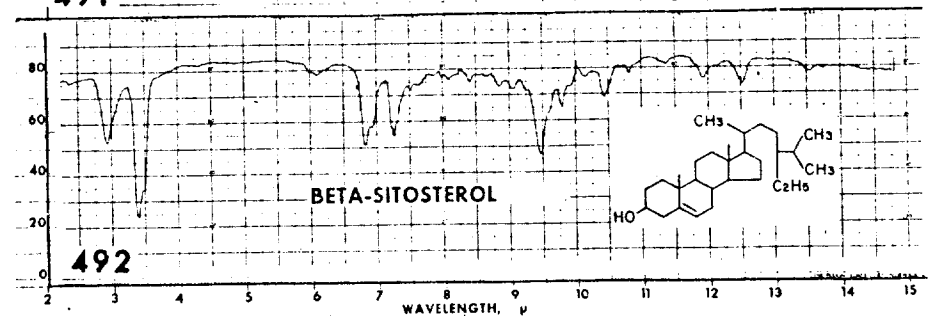


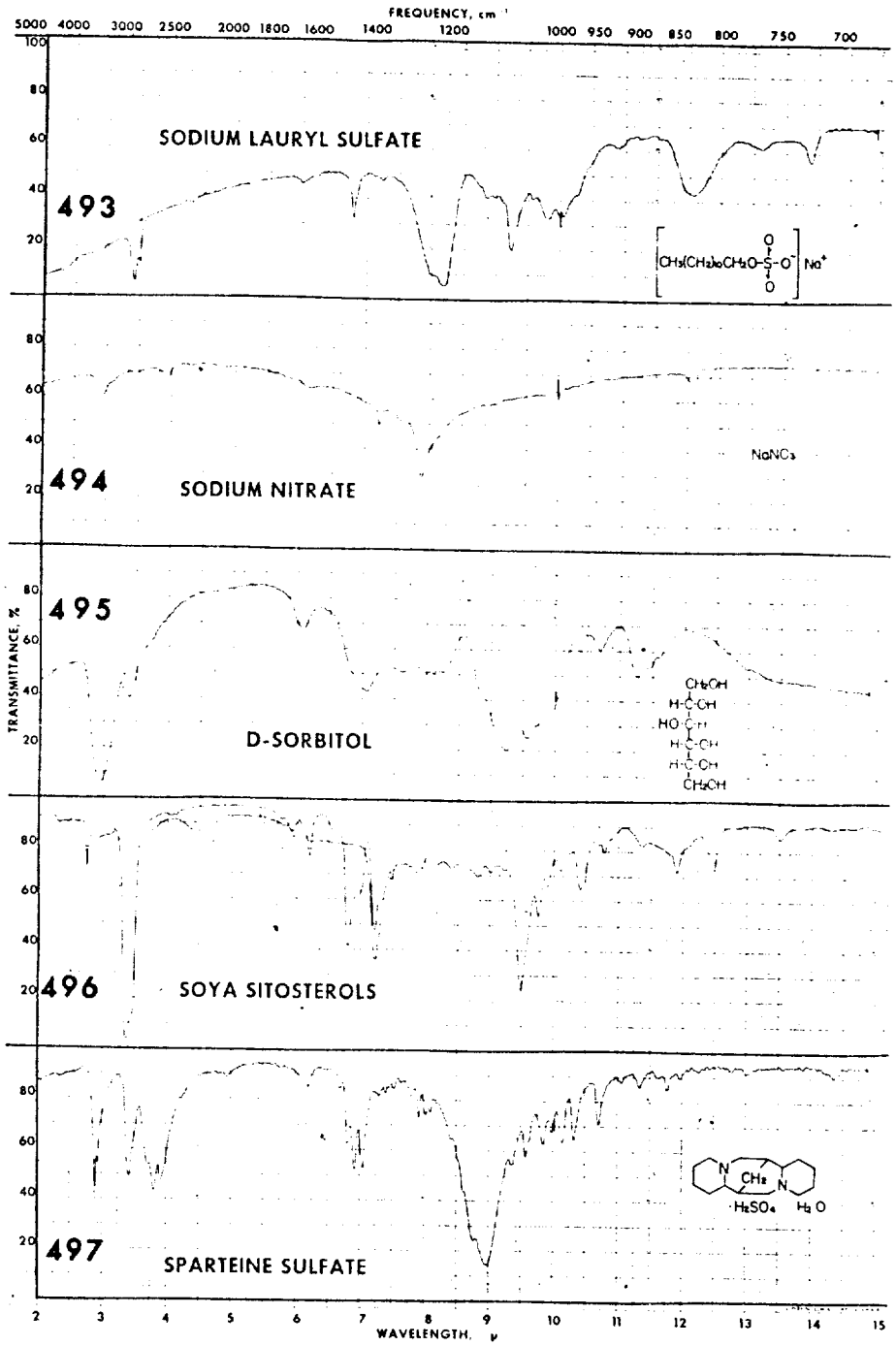


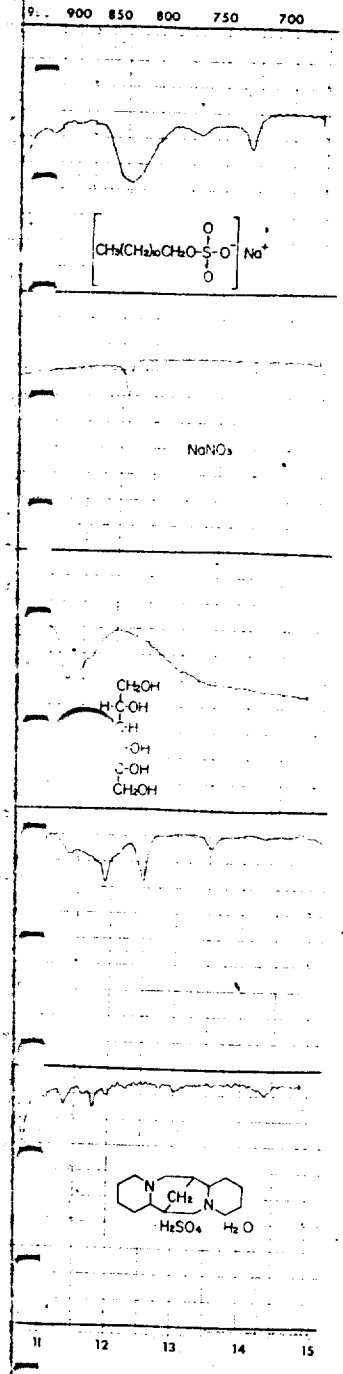


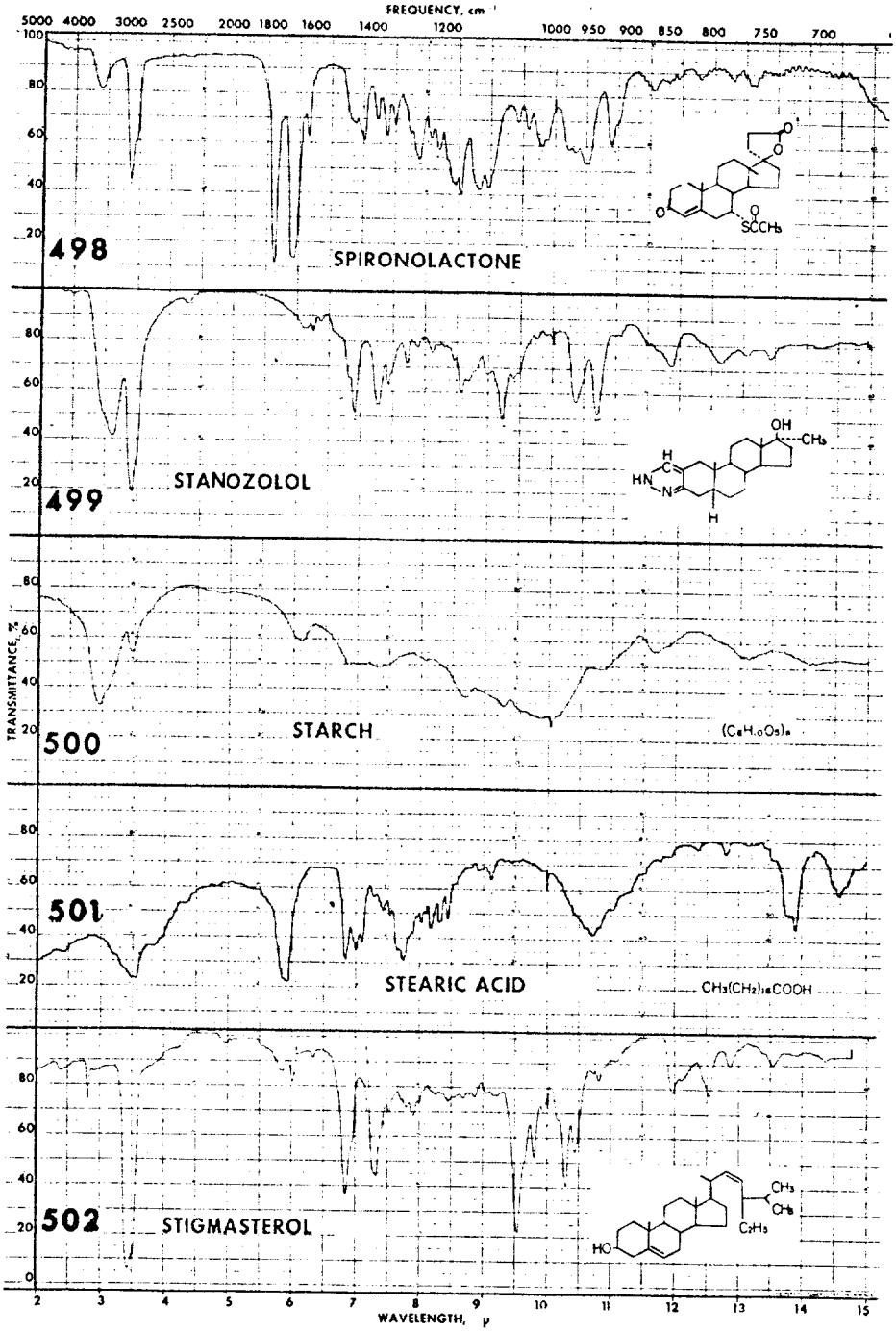


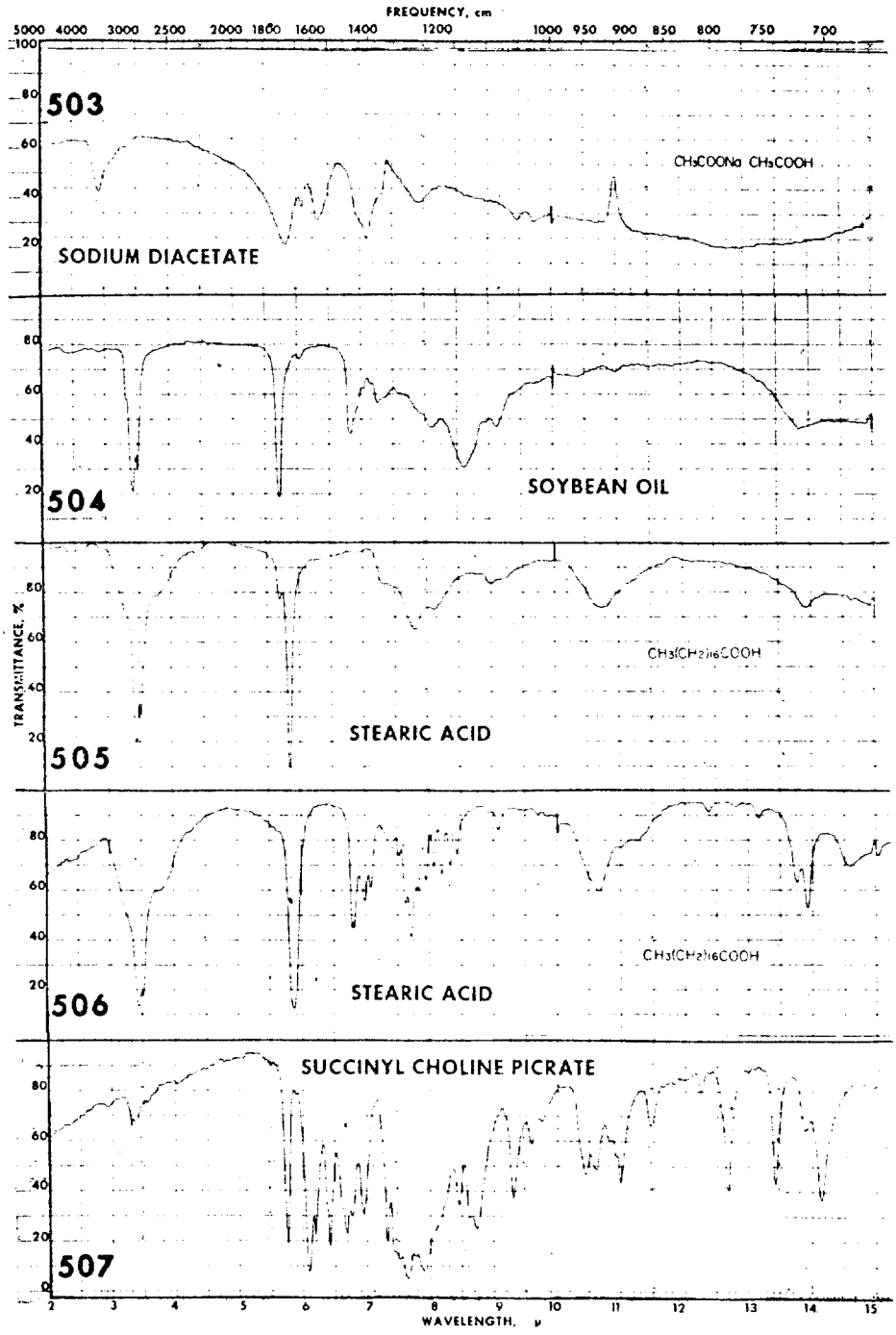





FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
493
SODIUM LAURYL SULFATE
494
SODIUM NITRATE
NaNO3
495
D-SORBITOL
496
SOYA SITOSTEROLS
497
SPARTEINE SULFATE
TRANSMITTANCE, %
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15


900 850 800 750 700
NaNO₃
11 12 13 14 15


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
498 SPIRONOLACTONE
499 STANOZOLOL
500 STARCH
501 STEARIC ACID
CH₃(CH₂)₁₆COOH
502 STIGMASTEROL
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
503
CH3COONa CH3COOH
SODIUM DIACETATE
504
SOYBEAN OIL
TRANSMITTANCE, %
505
CH3(CH2)16COOH
STEARIC ACID
506
CH3(CH2)16COOH
STEARIC ACID
SUCCINYL CHOLINE PICRATE
507
WAVELENGTH, μ

950 900 850 800 750 700

$CH_3COONa \cdot CH_3COOH$

OYBEAN OIL

$CH_3(CH_2)_{16}COOH$

$CH_3(CH_2)_{16}COOH$

...IE

0 11 12 13 14 15

FREQUENCY, cm^{-1}

5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700

100 80 60 40 20

SUCROSE

508

SULFACHLOROPYRIDAZINE

509

TRANSMITTANCE, %

SULFAMETHIZOLE

510

SULFAPHENAZOLE

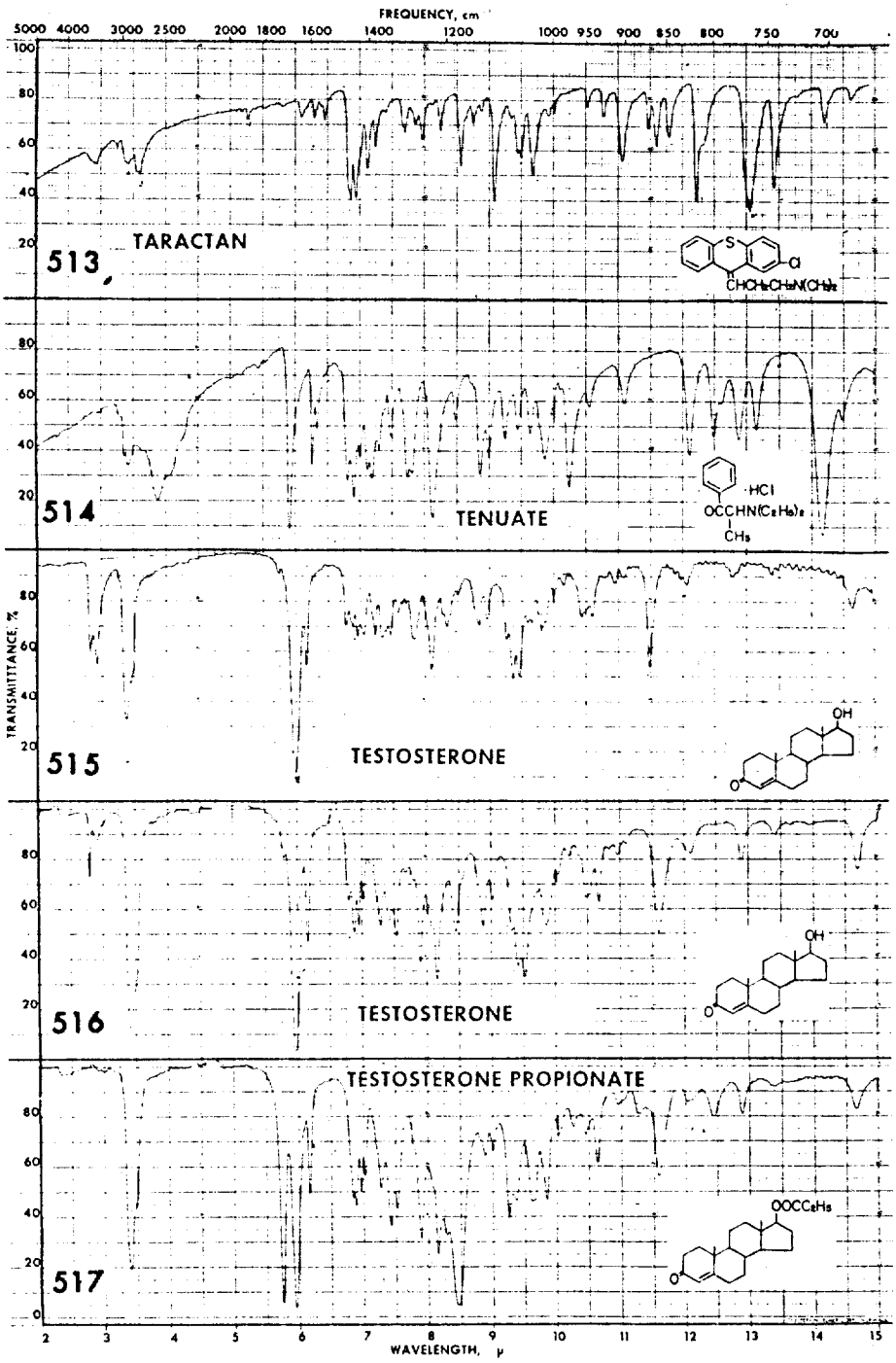
511

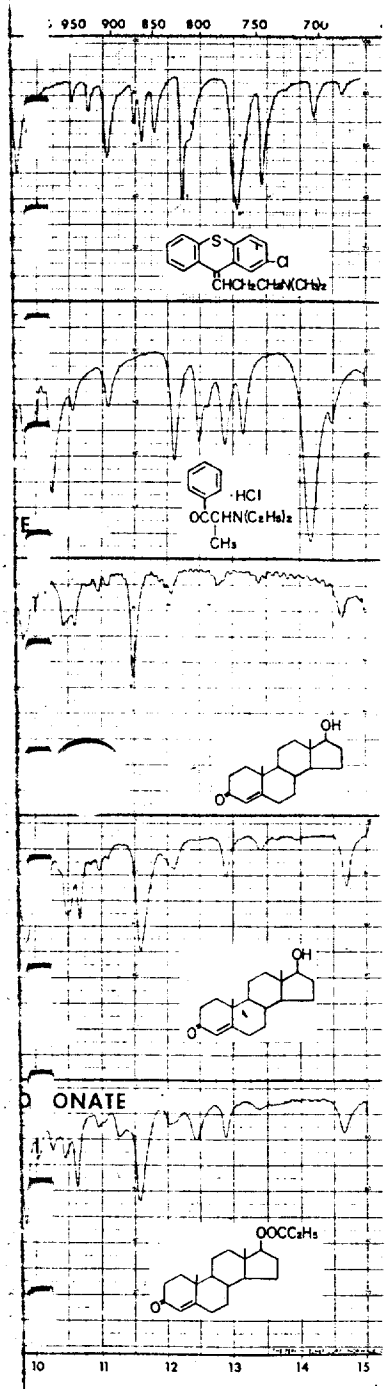
SULFOBROMOPHTHALEIN

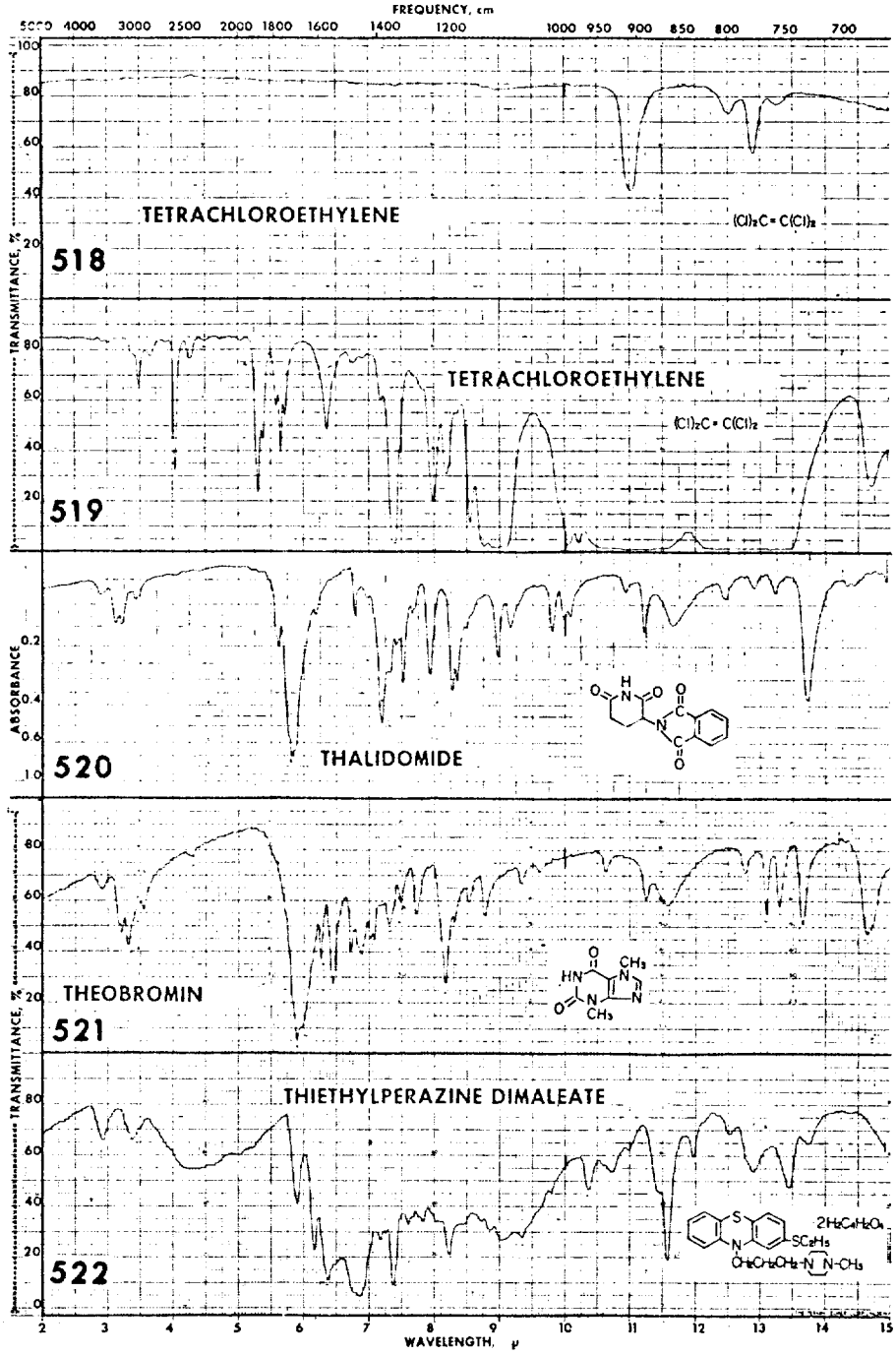
512

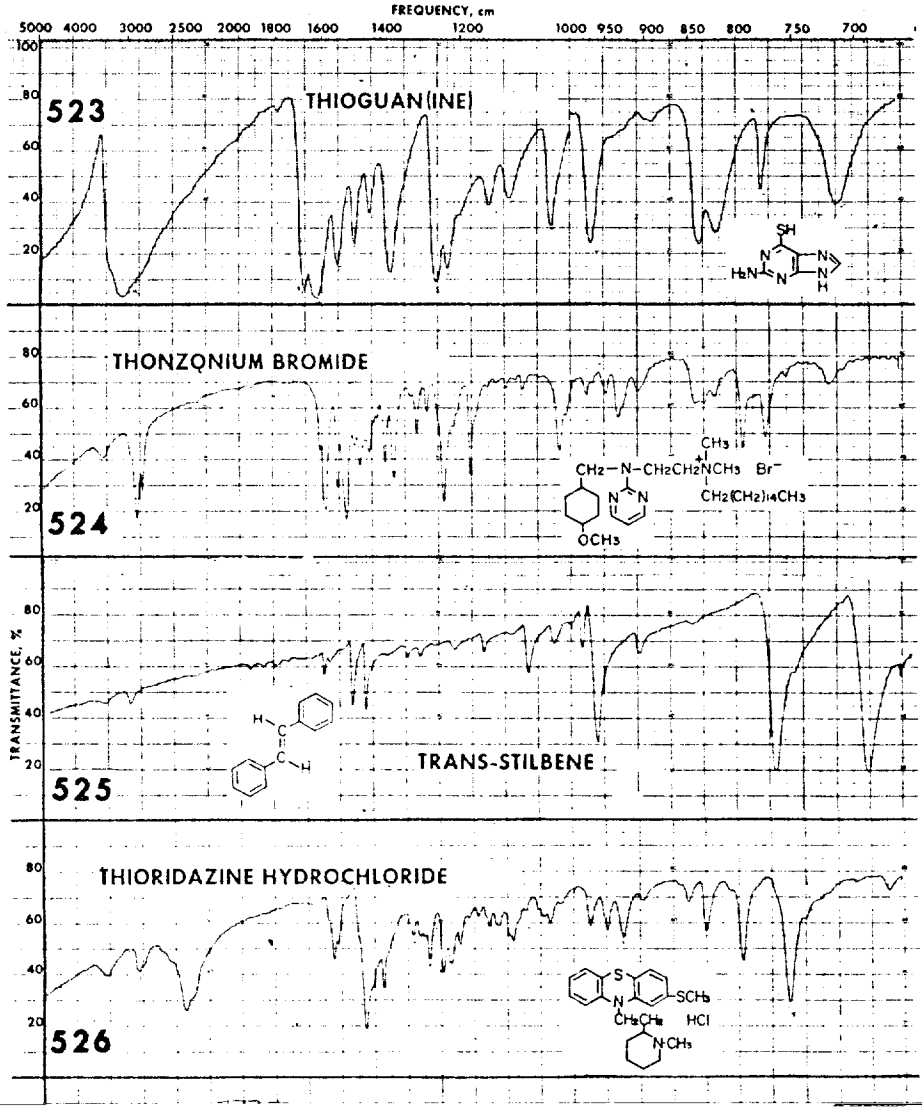
2 3 4 5 6 7 8 9 10 11 12 13 14 15

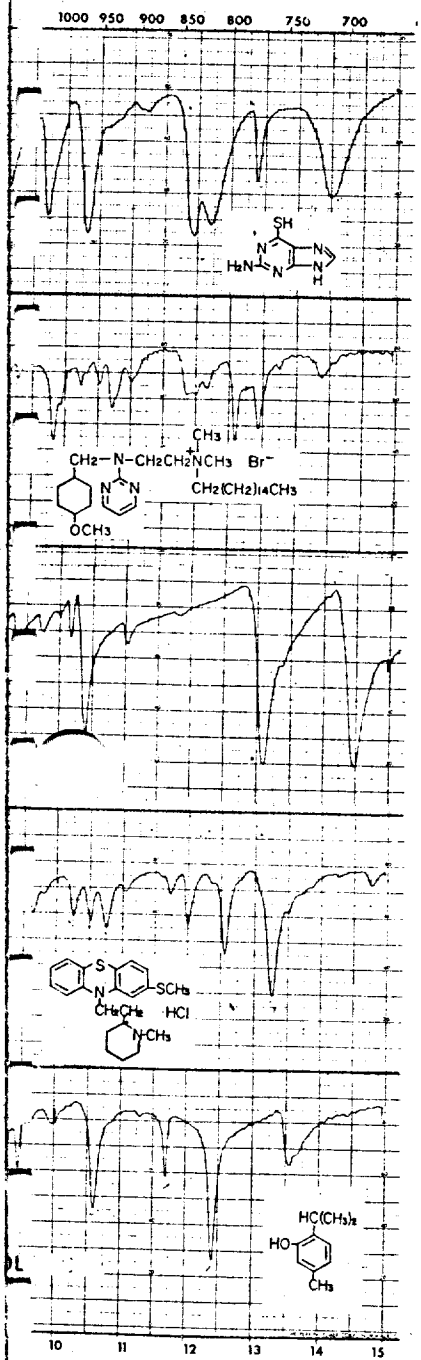
WAVELENGTH μ

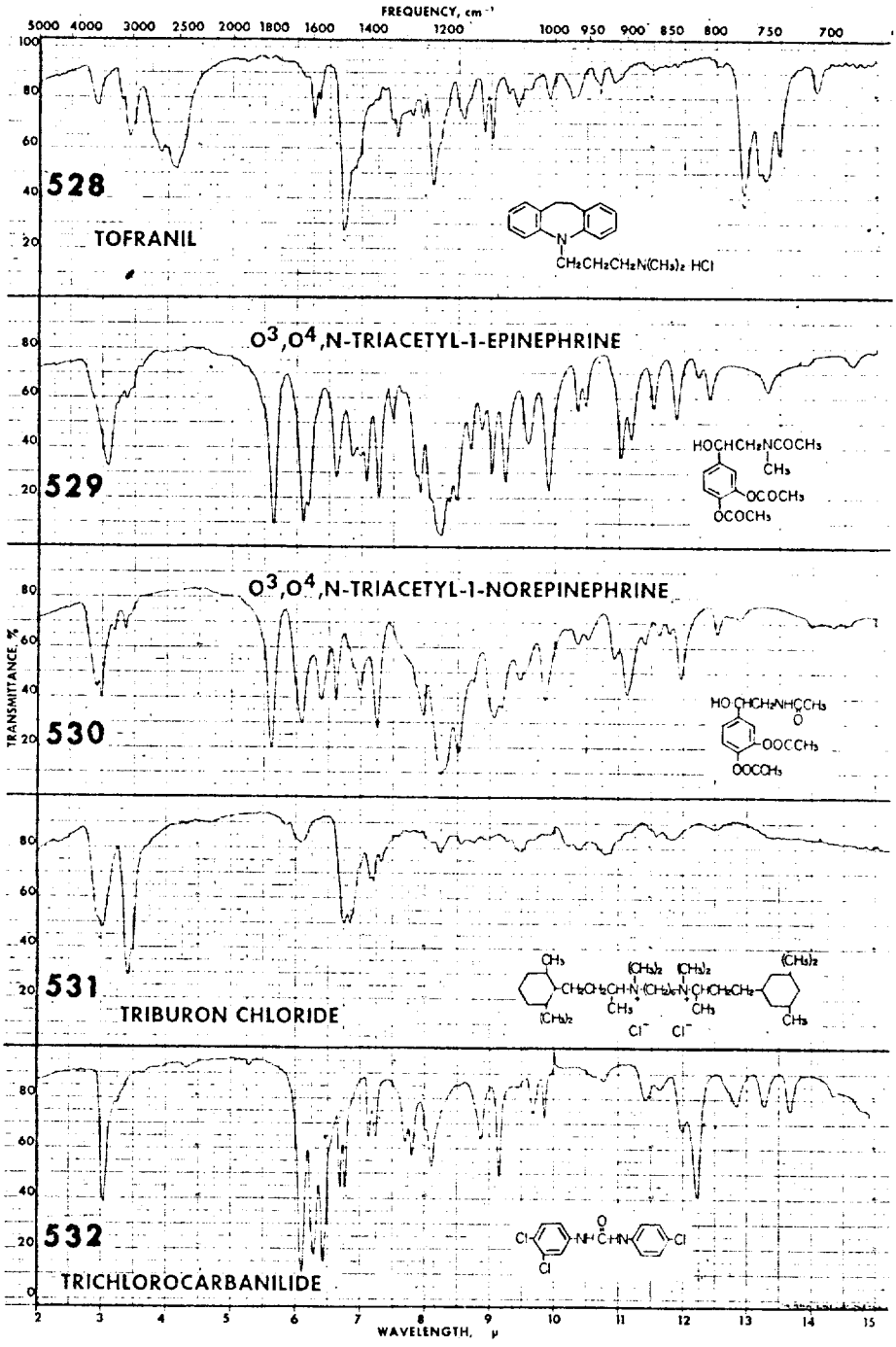

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
513 TARACTAN
514 TENUATE
515 TESTOSTERONE
516 TESTOSTERONE
517 TESTOSTERONE PROPIONATE
WAVELENGTH, μ
2 3 4 5 6 7 8 9 10 11 12 13 14 15

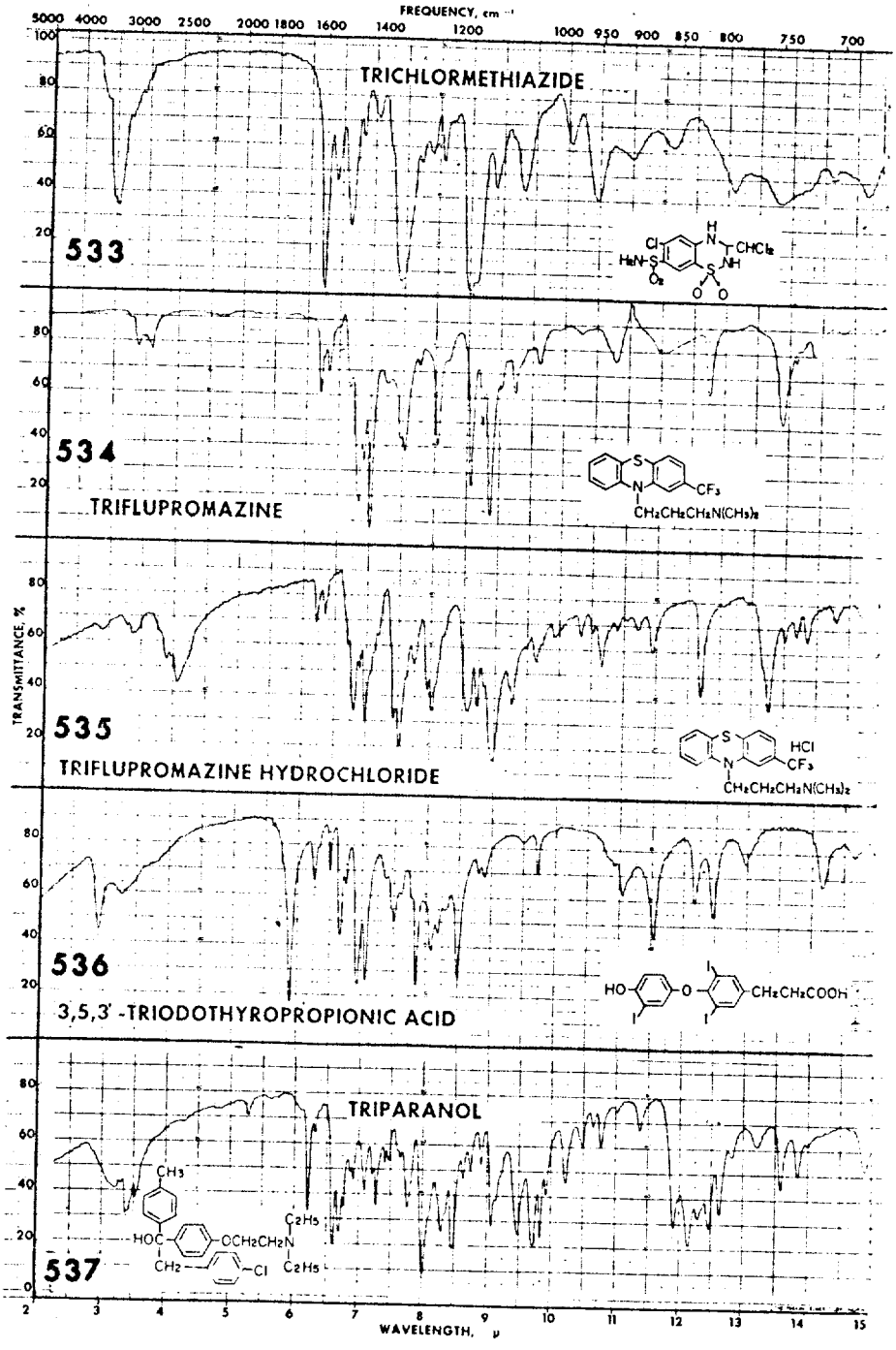

950 900 850 800 750 700
HCl
ONATE
10 11 12 13 14 15

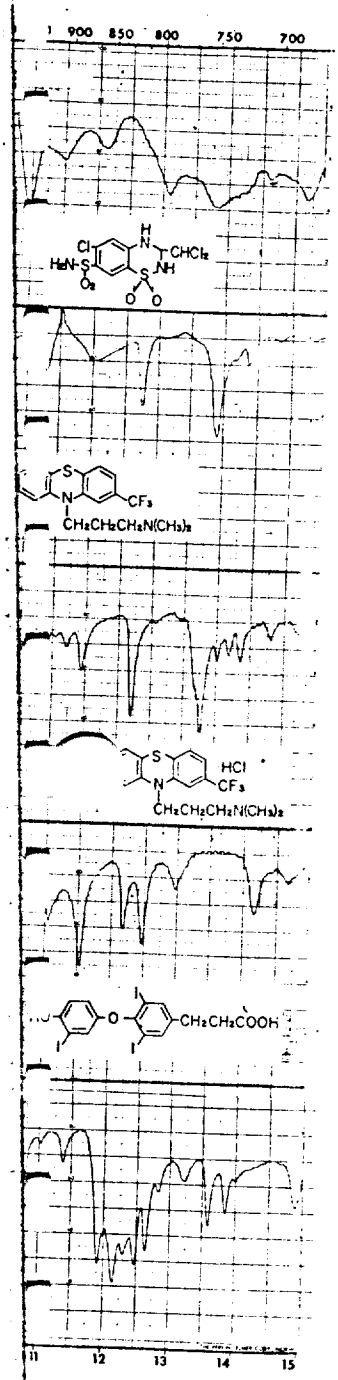

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
TETRACHLOROETHYLENE
518
519
ABSORBANCE
THALIDOMIDE
520
THEOBROMIN
521
THIETHYLPERAZINE DIMALEATE
522
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

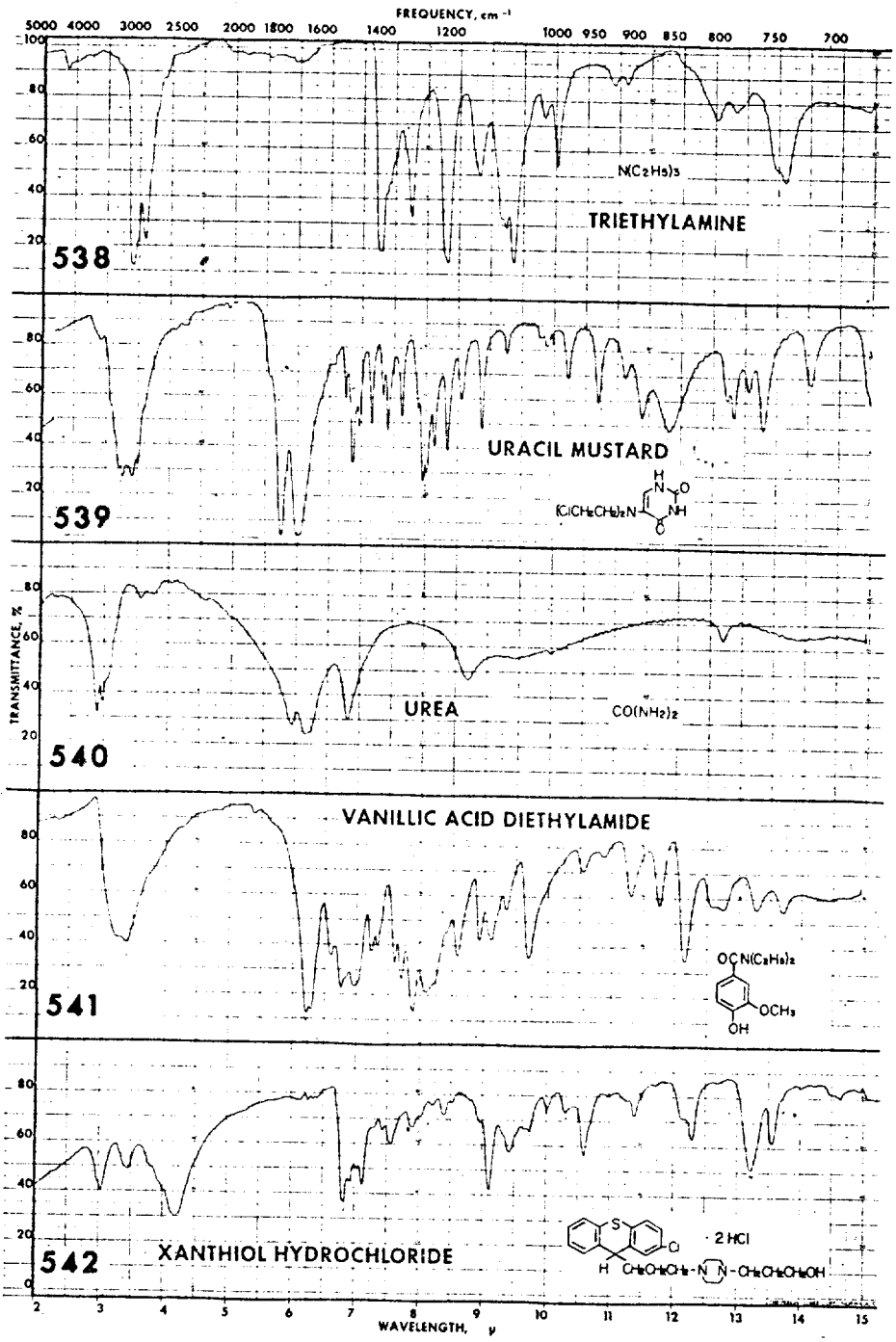

FREQUENCY, cm
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
523
THIOGUANINE
524
THONZONIUM BROMIDE
525
TRANS-STILBENE
526
THIORIDAZINE HYDROCHLORIDE


1000 950 900 850 800 750 700
10 11 12 13 14 15


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE, %
528
TOFRANIL
529
O³,O⁴,N-TRIACETYL-1-EPINEPHRINE
530
O³,O⁴,N-TRIACETYL-1-NOREPINEPHRINE
531
TRIBURON CHLORIDE
532
TRICHLOROCARBANILIDE
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
TRANSMITTANCE %
533
TRICHLORMETHIAZIDE
534
TRIFLUPROMAZINE
535
TRIFLUPROMAZINE HYDROCHLORIDE
536
3,5,3'-TRIODOTHYROPROPIONIC ACID
537
TRIPARANOL
WAVELENGTH, μ


1 900 850 800 750 700
11 12 13 14 15


FREQUENCY, cm⁻¹
5000 4000 3000 2500 2000 1800 1600 1400 1200 1000 950 900 850 800 750 700
N(C2H5)3
TRIETHYLAMINE
538
URACIL MUSTARD
539
TRANSMITTANCE, %
UREA
CO(NH2)2
540
VANILLIC ACID DIETHYLAMIDE
541
XANTHIOL HYDROCHLORIDE
542
2 HCl
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH, μ

Am J Hygiene 16: (865-869) (1932)

A MICROSCOPIC STUDY OF THE TISSUES OF THE ALBINO RAT FOLLOWING THE INGESTION OF ALUMINUM SALTS.*

By

ERNEST SCOTT AND MARY K. HELZ

(Received for publication April 29, 1932.)

In addition to the animals used in the present series of experiments this study includes a complete histological review of tissues of those rats fed sodium aluminum sulfate baking powder by Lyman and Scott (1) in their work on The Effects of the Ingestion of Tartrate or Sodium Aluminum Sulfate Baking Powders upon the Growth, Reproduction and Kidney Structure in the Rat.

The animals used in the experiments of Scott and Lyman were fed a basal ration of:

Wheat meal	6 parts
Poultry meat scrap	1 part
Dried skim milk	1 part
Lard or butter	2 parts
Salt	0.1 part

Group 1, the control group, received only this basal diet. Group 2 received the control diet plus one gram of the baking powder to 212 grams of food. Group 3 received the control diet plus one gram of the baking powder to 53.5 grams of food. Group 4 received the control diet plus one gram of the baking powder to 636 grams of food. In addition to this diet the animals were fed about 0.5 grams of cod liver oil per rat per week and also green stuff in season.

The initial weight of the animals in all groups was between 50 and 60 grams. Since it appears that the rate of growth varies slightly with sex, the two sexes were separated. The average time, in days, required by eight control males to gain 100 grams was 47. The average time for 26 of the aluminum fed male animals, representing all groups, to make the same gain was 15.1 days. The average time required for four females of the control group to gain 80 grams was 40 days. The average time for 20 of the aluminum fed females to make

* From the Department of Pathology, Ohio State University.

the same gain was 37.6 days. In this experiment it seems that a more rapid initial growth occurred in those animals which received aluminum salts in addition to their food. This increase in growth is not varied by the amounts of aluminum used.

The authors state in their summary that "varying amounts of sodium aluminum sulfate baking powder up to approximately 2 per cent of the diet have no appreciable effect on the rate of growth, maximum adult size, longevity, reproduction and non-protein-nitrogen of the blood."

The kidneys of the rats above described, some of which had been fed for a period of 21 months on as high as 2 per cent S.A.S. baking powder, presented neither gross nor microscopic lesions whereby they could be distinguished from the kidneys of the control group.

In the present report the kidneys of the animals used in the previously described experiments were reexamined and the examination extended to include the remaining organs of these animals. This report also includes two other series of animals which were fed much larger doses of aluminum than those of the previous groups. In this latter work the chemically pure chloride of aluminum was used. This discussion considers 21 test and 4 control animals reported by Scott and Lyman, 8 animals fed 3.6 per cent aluminum chloride for over a year, and also 5 test and 6 control animals receiving 3.6 per cent aluminum chloride for 57 days.

These latter groups of animals were started when 3 to 4 weeks of age and averaged 40 grams in weight. The basic ration for both control and test animals consisted of a "growing mash" used for baby chicks. This was a well balanced feed put out by a local elevator and has proven fully adequate for growth and reproduction in our other laboratory animals. The test group received this mash plus 3.6 per cent aluminum chloride ($AlCl_3.6H_2O$). This was thoroughly mixed in a mortar and fed dry. The control group received only the basic ration.

The day before they were killed the animals were weighed, the aluminum fed group averaging 204 grams and the controls averaging 191 grams. Thus at the end of the 57 day period the aluminum fed rats had gained more weight than the controls. This is in strict accord with the findings of Scott and Lyman as well as with those of Myers and Mull (2), McCollum, Rask and Becker (3), Massatsch (4), Rose and Catherwood (5), and Dee Tourtellotte and Rask (6), but at variance with those of Schaeffer et al. (7).

On the day preceding the killing of the animals complete blood

counts and hemoglobin determinations were made on all animals of the aluminum fed group and on three of the control group.

Test.

Rat no.	Dare HB. per cent	Red cells	White cells
1	80	7,650,000	12,100
2	80	7,650,000	18,400
3	110	9,980,000	21,500
4	95	9,000,000	23,000
5	90	9,800,000	16,500
		Control.	
6	95	8,600,000	9,800
7	100	10,000,000	12,000
8	110	9,400,000	13,200

Two of the aluminum fed rats showed a slightly low hemoglobin and red cell count, though this variation is still well within the normal limits set by Donaldson (8).

It is to be recalled that these animals were receiving huge doses of the aluminum salt in comparison with the amounts consumed in normal human intake. Bulletin no. 103 of the United States Department of Agriculture (17) considers 150–200 mg. of aluminum per day a large dose for an adult human being, the average amount consumed by individuals being from 25–75 mg. per day for the days in which such articles are consumed. Dr. E. E. Smith has figured this average intake to be equal to approximately 1 mg. per day per kilo body weight. It is possible that this mild anemia and leucocytosis might be explained by the administration of such excessive amounts of aluminum.

Waltner and Waltner (9), in their work, found that metallic aluminum brought about a decrease in hemoglobin and red cells; Mitchell and Miller (10), in their experiments in nutritional anemias found that salts of aluminum, when added to an anemia-producing diet, had absolutely no effect upon the animals. Seibert and Wells (11) fed rabbits small doses of aluminum salts and at the same time injected a solution of the same salt into the blood stream. They record definite anemia as the result of their treatment. The slight decrease of hemoglobin and red blood cells recorded in two of our animals, which is not accompanied by any gross or microscopic pathology, may possibly be attributed to an acidosis. It is generally conceded that when feeding the metallic chlorides in large doses, the acid ion is absorbed more

rapidly than the basic ion. This might give rise to an acidosis and an accompanying mild anemia. It is conceded that rats are much more resistant to acidosis than rabbits. Another factor which would make the results in the two experiments incomparable is the fact that Seibert and Wells made injections of the salt into the blood stream simultaneous with its feeding.

Chemical determinations for iron content were made upon the livers of the test, and of the control animals used in the last experiment. The combined livers of the 6 control animals weighed 38 grams and contained 1.83 mg. of iron or 4.81 mg. iron per 100 gm. of liver. The combined livers of the 5 aluminum chloride fed rats weighed 50 grams. The total iron content of the livers of these animals was 2.48 mg. of iron or 4.96 mg. iron per 100 grams of liver. Thus a normal content of iron was found in the livers of both the test and the control animals.

Macroscopic examination of the organs of these animals including the heart, both lungs, spleen, liver, both kidneys, both adrenals, cardiac and pylorie portions of the stomach, both testes and epididymes, ovaries, pancreas, duodenum, jejunum, caecum and colon, presented no gross pathological changes. It may be further stated that the microscopic examination of these same organs revealed no changes which could be interpreted as pathologic. The ovaries contained many follicles in all stages of maturity as well as numerous corpora lutea. The spleens of both the control and aluminum fed animals showed a moderate pigmentation. Pigmentation also occurred in other groups of animals fed upon entirely different diets. None of the other conditions noted by Seibert and Wells in their rabbits and attributed by them to the aluminum salts appeared in the spleens of the rats of this series.

Sections from the cardiac portion of the stomach of all rats fed aluminum salts appeared normal. The cardiac portion of the stomach of one *control* animal, however, showed several lesions, appearing as ulcerations. One such ulcer extended completely through the epithelium. In the base of these ulcers the submucosa was edematous and infiltrated with a mixture of polymuclear leucocytes. Throughout the remainder of the gastro-intestinal tract there were no inflammatory lesions present. There did occur, however, slight changes in the epithelium, which were interpreted as autolysis. The epithelium in these areas failed to stain and in a few instances sloughed off. This was not accompanied by any edema, congestion, or cellular exudation. The condition appeared as often in the control animals as in those fed aluminum compounds and it was also noticed in animals upon entirely different experimental diets. Autolytic changes occurred

only where the stomach or intestines contained partly digested food material. Animals which had fasted twenty-four hours previous to slaughter seldom showed this condition. We therefore found no lesions in the gastro-intestinal tract which could be attributed to the presence of aluminum. McCollum, Rask and Becker (12) feel that this should be the case, as they state that aluminum compounds do not form a union with the gastro-intestinal mucous membrane. These findings are substantiated by Dee Tourtellotte and Rask in rats fed 0.6 per cent aluminum chloride. These animals appeared healthy, normal in size, reproduction, general appearance and in the gross appearance of the livers, spleens, hearts, and kidneys. Conclusions based upon the foregoing observations do not support those of Schaeffer and his co-workers (13–7), who feel that the presence of aluminum chloride in the stomach might give rise to gastric and duodenal ulcers even in as small amounts as would be present from the daily use of aluminum-containing baking powders, and who describe (14) very definite inflammatory lesions in the gastro-intestinal mucous membrane of both mice and dogs. Ceriotti (15) in general agrees with Schaeffer in that aluminum chloride is not precipitated by the alkaline pancreatic juice and states that dogs fed a bread containing aluminum show slowing up of gastric evacuation with an accompanying irritation of the gastric mucosa. He also suggests that aluminum chloride formed in the stomach may be responsible for gastric ulcers in human beings.

The German Health Bureau (16) fed dogs large quantities of aluminum hydroxide (corresponding to 1000 mg. of aluminum oxide) daily over a period of twelve months. There was no change in the condition, appetite or body weight; neither were there any changes macroscopically or microscopically in the organs. This same experiment was conducted on humans without any apparent detriment to health or well-being. Neither was there any evidence that the aluminum was absorbed into the tissues or blood stream. Therefore these investigators conclude that the ingestion of large amounts of aluminum hydroxide has no ill effects upon the human organism.

Conclusions.

The conclusions reached in this study are based upon the findings in 80 test and 22 control animals which had received varying amounts of aluminum salts up to 3.6 per cent of feed by weight.

The protracted ingestion of aluminum salts in concentrations as high as 3.6 per cent has no deleterious effect upon the growth, reproduction, or blood picture of the white rat.

Livers of the animals examined contained a normal amount of iron.

There was no evidence of gross or microscopic pathology in the organs examined which could be attributed to the ingestion of aluminum salts. Autolytic changes in the stomach and intestine occurred only where they contained partly digested food material. Animals which had fasted twenty-four hours previous to slaughter seldom showed this condition. We therefore found no lesions in the gastro-intestinal tract which can be attributed to the presence of aluminum.

We are indebted to Dr. J. F. Lyman of the Department of Agricultural Chemistry of the Ohio State University for the determinations of iron in the livers of these animals.

Bibliography.

1. LYMAN, J. F., AND SCOTT, ERNEST.
1930. Amer. Jour. Hyg., *12*, 271–282.
2. MYERS AND MULL.
1928. Jour. Biol. Chem., *78*, 605–13.
3. MCCOLLUM, RASK, AND BECKER.
1928. Jour. Biol. Chem. *77*, 753–68.
4. MASSATSCH, C.
1929. Hauszeitschrift, Berlin, June 1.
5. ROSE, W. C., AND CATHERWOOD, FLORENCE.
1929. Jour. Nutrition, *2*, November, 155–69.
6. DEF TOURTELLOTTE, AND RASK, O. S.
1931. Amer. Jour. Hyg., *14*, 228.
7. SCHAEFFER, G., ET AL.
1928. Bulletin Soc. sc. d'hyg. aliment., *16*, 1–24; 49–79
8. DONALDSON, HENRY H.
1924 The Rat. Philadelphia.
9. WALTNER, K., AND WALTNER, K.
1929 Arch. exp. Path. u. Pharmakol., *136*, 310–2.
10. MITCHELL, H. S., AND MILLER, L.
1931. Jour. Biol. Chem., *92*, 423.
11. SEIBERT, FLORENCE B., AND WELLS, H. GIDEON.
1929. Arch. Path., *8*, 230–62.
12. MCCOLLUM, RASK, AND BECKER.
1929. Bull. Soc. sc. d'hyg. aliment., *17*, 65–73.
13. SCHAEFFER, FONTES, AND OBERLING.
1929. Bull. Soc. sc. d'hyg. aliment., *17*, 74–87.
14. SCHAEFFER ET AL.
1928 Jour. of Hyg., *28*, August, 92–99.
15. CERECETO, ANTONIO.
1930 Rev. facultad. cienc. quim. Univ. La Plata), *6*, 51–3.
16. Reichs-Gesundheitsblatt herausgegeben vom Reichsgesundheitsamt, Berlin, *5*, no. 45, 865 1930.
17. Bulletin U. S. Dept. Agr. no. 105, 6.

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH KASAL IN ALBINO RATS

JUNE 28, 1972

IBT NO. B747

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH
KASAL
IN ALBINO RATS

JUNE 28, 1972

IBT NO. B747

I. Introduction

A sample identified as Kasal was received from the Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of albino rats fed Kasal at dietary levels of 0.3, 1.0 and 3.0 percent. Results obtained from microscopic examination of tissues and organs disclosed microconcretions in the renal tubules of the female rats from all three test groups. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related. No abnormalities were observed in any of the following parameters:

Body Weight Gains
Food Consumption
Hematologic Studies
Clinical Blood Chemistry Studies
Urine Analyses
Gross Pathologic Studies
Organ Weights and Ratios

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Philip S. Smith

Philip S. Smith, B.S.
Assistant Toxicologist
Rat Toxicity

Report approved by:

James B. Plank

James B. Plank
Senior Group Leader
Rat Toxicity

Paul L. Wright

Paul L. Wright, Ph.D.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

slg

Stauffer Chemical Company
Industrial Chemical Division

CERTIFICATE OF ANALYSIS

Material: Sodium Aluminum Phosphate, Basic Date 10/22/71
Common Name: Kasal

Identification: Material representing 20 bags Kasal coded 140-14230
Lot K-108

Analysis:

Determination		Food Chemical Codex Specifications
Assay (Al_2O_3)	10.9%	9.5% - 12.5%
Loss on Ignition	8.02%	9% maximum
Limits of Impurities		
Arsenic (As)	0.1 ppm	3 ppm maximum
Fluoride (F)	2.0 ppm	25 ppm maximum
Heavy Metals (as Pb)	<10 ppm	40 ppm maximum
Lead (Pb)	0.3 ppm	10 ppm maximum
P_2O_5	45.6%	-
pH (25% slurry)	9.2	-
Sieving:		
on 200 mesh	None	-
on 325 mesh	2.0%	-

This material complies with the specification of the Food Chemicals Codex.

R.S. Bryant

STATE OF NEW YORK
COUNTY OF NEW YORK

Sworn and subscribed to before me this
22nd day of October, 1971.

Patricia A. Pecquet

PATRICIA A. PECQUECK
NOTARY PUBLIC, State of New York
No. 24-6312590
Qualified in Kings County
Cert. filed in New York County
Commission Expires March 30, 1972

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. One hundred and twenty rats (60 males and 60 females) were selected for the experiment and housed individually in standard, wire-bottomed steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, individual animal number and sex.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Outline of Experiment

Group	Number of Animals Males	Females	Dietary Level (percent)
Control	15	15	None Administered
T-I	15	15	0.3
T-II	15	15	1.0
T-III	15	15	3.0

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at weekly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period and the data subjected to statistical analyses.

D. Food Consumption and Diet Preparation

Food consumption data were collected individually for five rats of each sex in every group weekly during the study and the data recorded.

The diet for any given group was prepared by blending the appropriate amount of Kasal with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one weeks' ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Hematologic, Clinical Blood Chemistry Studies and Urine Analyses

Blood and urine samples collected individually from ten rats of each sex from both the control and T-III groups after 45 and 84 days of feeding were analyzed for the following:

1. Hematologic Studies

   a. Hematocrit Value
   b. Erythrocyte Count
   c. Hemoglobin Concentration
   d. Total Leukocyte Count
   e. Differential Leukocyte Count

2. Clinical Blood Chemistry Studies

   a. Blood Urea Nitrogen (BUN) Concentration
   b. Serum Alkaline Phosphatase (SAP) Activity
   c. Serum Glutamic-Pyruvic Transaminase (SGPT) Activity
   d. Fasted Blood Glucose Concentration

3. Urine Analyses

   a. Glucose Concentration
   b. Albumin Concentration
   c. Microscopic Elements Examination
   d. pH
   e. Specific Gravity

G. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of gross examination a complete set of organs and other tissues was removed from each rat and preserved in formalin solution. Also at autopsy the weights of the liver, kidneys, spleen, gonads, heart and brain of each rat were determined and recorded.

Microscopic examination of tissues taken from ten rats of each sex from both the control and T-III groups was conducted. The following tissues, stained with Hematoxylin-Eosin, were included: esophagus,

stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate gland, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

H. Organ Weights, Organ to Body Weight and Organ to Brain Weight Ratios

Statistical analyses were conducted upon the absolute organ weights and their corresponding ratios to the weight of the body and brain. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Statistical comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (percent)	Sex	Body Weight (grams) Week: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Weight Gain (grams/rat)
Control	M	185	242	298	349	361	391	417	437	458	481	515	518	521	522	337
	F	150	185	211	232	245	253	260	275	281	291	304	301	318	331	181
0.3	M	187	244	300	347	376	408	445	469	482	501	524	524	535	546	359
	F	149	190	214	234	245	260	274	280	289	297	307	299	318	330	181
1.0	M	185	247	310	353	382	411	442	464	474	499	524	527	542	558	373
	F	147	176	201	220	231	239	252	260	265	272	294	284	288	291	144
3.0	M	185	235	287	343	372	406	428	451	467	493	513	521	533	545	360
	F	150	176	210	227	233	251	260	263	271	277	285	284	291	300	150

B. Food Consumption

Food consumption data collected during the 90-day test period are summarized in Table III.

Test rats ate amounts of food comparable to that consumed by control rats.

TABLE III

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Food Consumption Data

Summary of Mean Values

Dietary Level (percent)	Sex	Food Consumption (grams/rat/seven days) Week: 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption (grams/rat)
Control	M	154	148	169	163	191	204	124	206	185	201	186	191	174	2296
	F	108	117	125	117	147	149	97	148	160	128	117	127	165	1705
0.3	M	148	152	154	180	180	200	141	191	188	182	120	182	176	2194
	F	147	107	119	140	135	144	91	115	130	131	113	138	145	1655
1.0	M	143	139	148	200	139	185	141	183	174	164	181	203	181	2181
	F	102	112	101	162	101	143	101	134	121	124	115	132	137	1585
3.0	M	116	175	161	223	164	214	142	192	194	192	133	182	165	2253
	F	104	104	105	166	113	139	87	162	123	119	117	133	152	1624

C. Mortality and Reactions

Three deaths occurred during the study. One of these deaths was ascribed to an acute respiratory infection, while the other two resulted from trauma incurred during the collection of blood samples.

No untoward behavioral reactions were noted among any of the animals employed in the study.

D. Hematologic Studies

The results of the hematologic studies conducted on blood samples taken from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables IV and V.

No outstanding differences between test and control rats were noted with respect to any of the parameters investigated.

TABLE IV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Total Leukocyte Count (thousands/mm^3) Day: 45	Total Leukocyte Count (thousands/mm^3) Day: 84	Erythrocyte Count (millions/mm^3) Day: 45	Erythrocyte Count (millions/mm^3) Day: 84	Hemoglobin Concentration (g/100 ml) Day: 45	Hemoglobin Concentration (g/100 ml) Day: 84	Hematocrit Value (%) Day: 45	Hematocrit Value (%) Day: 84
Control	M	17.1	15.9	7.72	8.08	15.7	15.9	39.3	40.5
	F	14.7	9.9	7.70	7.52	15.5	15.7	38.4	37.9
3.0	M	15.7	16.0	7.83	7.98	15.7	15.7	39.1	39.6
	F	15.2	9.4	7.62	7.59	15.5	15.8	38.8	39.2

TABLE V

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Differential Leukocyte Count (Number of Cells per Hundred) Lymphocytes Day: 45	84	Neutrophils Day: 45	84	Monocytes Day: 45	84	Eosinophils Day: 45	84	Basophils Day: 45	84
Control	M	90.6	86.7	8.6	11.1	0.8	1.7	0.0	0.5	0.0	0.0
	F	83.2	85.5	15.2	12.6	0.8	1.0	0.8	0.9	0.0	0.0
3.0	M	87.6	84.1	10.6	12.8	1.2	2.0	0.6	1.1	0.0	0.0
	F	88.4	86.6	9.4	11.3	1.8	1.3	0.4	0.8	0.0	0.0

E. Clinical Blood Chemistry Studies

The results of the clinical chemistry studies conducted on blood samples obtained from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables VI and VII.

Values for test rats were not different from those of control rats.

TABLE VI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Serum Alkaline Phosphatase Activity (King-Armstrong Units) Day: 45	Day: 84	Serum Glutamic-Pyruvic Transaminase Activity (Dade Units) Day: 45	Day: 84
Control	M	31	24	30	27
	F	20	12	24	28
3.0	M	30	19	24	27
	F	18	17	24	25

TABLE VII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Blood Urea Nitrogen Concentration (mgs %) Day: 45	84	Fasted Blood Glucose Concentration (mgs %) Day: 45	84
Control	M	16	15	129	141
	F	17	14	130	137
3.0	M	17	15	150	131
	F	19	16	143	128

F. Urine Analyses

The results of the periodic examinations of urine specimens collected from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Table VIII.

No significant differences between the urine of the test rats and control rats were observed.

TABLE VIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Urine Analysis Data

Summary of Mean Values

Dietary Level (percent)	Sex	Glucose Day: 45	Glucose Day: 84	Albumin Day: 45	Albumin Day: 84	Microscopic Elements Day: 45	Microscopic Elements Day: 84	pH Day: 45	pH Day: 84	Specific Gravity Day: 45	Specific Gravity Day: 84
Control	M	n	n	t	n	+1	+2	7.0	6.8	1.025	1.039
	F	n	n	n	n	+2	+1	7.8	6.8	1.027	1.036
3.0	M	n	n	n	t	+1	t	7.0	7.4	1.030	1.043
	F	n	n	n	n	+1	+1	6.8	7.0	1.025	1.030

Glucose and Albumin

n = negative
t = trace; less than 30 mg/100 ml urine
+1 = 30 to 100 mg/100 ml urine
+2 = 100 to 300 mg/100 ml urine
+3 = 300 to 500 mg/100 ml urine
+4 = more than 500 mg/100 ml urine

Microscopic Elements

N = normal
t = minimal or trace amounts
+1 = slight amounts
+2 = moderate amounts
+3 = large amounts
+4 = extreme amounts

G. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Organ Weight and Organ to Body and Organ to Brain Weight Ratio Data

The results of the statistical analyses conducted on absolute organ weights, organ to body weight and organ to brain weight ratios are summarized in Tables IX through XIV.

Significant differences between a test group and the control group are designated by asterisks following the test values.

The number of statistically significant intergroup differences which were noted was considered to be normal for a random population of albino rats. The lack of any consistent dose or sex related response and the absence of any deleterious histopathologic changes further substantiate that none of the intergroup differences were related to the ingestion of Kasal.

TABLE IX

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - LIVER

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	18.140	10.064	3.4790	3.2526	8.8872	5.0896
0.3	20.853*	11.633*	3.8236	3.8286**	9.8207	6.2497**
1.0	19.413	9.325	3.4787	3.2024	9.2459	4.8121
3.0	18.462	9.485	3.3903	3.1562	8.8323	4.8732

* STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95 PERCENT CONFIDENCE LEVEL.
** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

TABLE X

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.666	2.117	0.7019	0.6798	1.8015	1.0695
0.3	3.755	2.184	0.6899	0.7212	1.7692	1.1753
1.0	3.937	1.993	0.7082	0.6865	1.8755	1.0274
3.0	3.971	2.375	0.7308	0.7916**	1.8971	1.2197*

* STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95 PERCENT CONFIDENCE LEVEL.
** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

TABLE XI

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - SPLEEN

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	0.936	0.618	0.1805	0.2031	0.4599	0.3087
0.3	0.814	0.589	0.1504	0.1943	0.3845	0.3173
1.0	0.887	0.524	0.1604	0.1808	0.4230	0.2701
3.0	1.004	0.660	0.1849	0.2186	0.4843	0.3419

O STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XII

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - GONADS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.611	0.077	0.6924	0.0253	1.7724	0.0392
0.3	3.607	0.100	0.6654	0.0332	1.7009	0.0542
1.0	3.527	0.089	0.6355	0.0309	1.6773	0.0465
3.0	3.487	0.080	0.6414	0.0269	1.6662	0.0418

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XIII

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - HEART

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	1.613	1.078	0.3095	0.3484	0.7937	0.5447
0.3	1.801*	1.083	0.3311	0.3581	0.8500	0.5829
1.0	1.785**	1.023	0.3217	0.3531	0.8498	0.5276
3.0	1.777*	1.081	0.3269	0.3605	0.8478	0.5558

* STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95 PERCENT CONFIDENCE LEVEL.
** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

TABLE XIV

TEST MATERIAL - KASAL

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - BRAIN

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	2.041	1.982	0.3914	0.6431	1.0000	1.0000
0.3	2.122	1.861**	0.3912	0.6203	1.0000	1.0000
1.0	2.109	1.938	0.3814	0.6736	1.0000	1.0000
3.0	2.097	1.953	0.3863	0.6535	1.0000	1.0000

** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

3. Histopathologic Findings

Histopathologic examination of tissues and organs taken from ten rats of each sex in both the control and T-III groups was conducted. Microscopic examination of sections of kidneys taken from ten rats of each sex from the T-II group and from all female rats from the T-I group was also conducted.

Tables XV through XVIII list all histopathologic changes noted.

IBT No. B747
Stauffer Chemical Company

I have completed a histopathologic evaluation of a series of rat tissues from IBT No. B747. There are microconcretions present in the renal tubules of the female rats from all three dose levels. These concretions are located in the tubules at the corticomedullary junction and they consist of an amorphous material which shattered on sectioning. They are blue in color and are probably calcified. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related.

The other lesions observed are those of spontaneous disease and they are not unusual for the albino rat.

Ward R Richter

Ward R. Richter, D.V.M., M.S.
Diplomate, American College of
Veterinary Pathologists

TABLE XV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: Control

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Chronic tracheitis	6	1.0
	Lung	Chronic murine pneumonia	3	1.0
	Colon	Parasites	1	1.0
	Kidney	Focal lymphoid infiltration	3	1.0
	Urinary bladder	Mucoid plug	6	1.0
10 Females	Lung	Chronic murine pneumonia	1	1.0
	Colon	Parasites	1	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-I (0.3%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
15 Females	Kidney	Microconcretions	4	0.5
		Chronic nephritis	2	2.0
		Focal lymphoid infiltration	1	1.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-II (1.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Kidney	Tubular nephrosis	1	1.0
		Chronic nephritis	1	1.0
10 Females	Kidney	Microconcretions	3	2.0
		Chronic nephritis	2	1.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-III (3.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Chronic tracheitis	2	1.0
	Lung	Chronic murine pneumonia	2	1.0
	Kidney	Focal interstitial nephritis	2	1.0
	Urinary bladder	Mucoid plug	3	1.0
	Brain	Focal encephalitis (nosematosis)	1	1.0
10 Females	Trachea	Chronic tracheitis	4	1.0
	Lung	Chronic murine pneumonia	1	1.0
	Kidney	Micronconcretions	8	2.0
		Focal lymphoid infiltration	1	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVAIR IN ALBINO RATS

JUNE 28, 1972

IBT NO. B747

32

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH
LEVAIR
IN ALBINO RATS

JUNE 28, 1972

IBT NO. B747

I. Introduction

A sample identified as Levair was received from the Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of albino rats fed Levair at dietary levels of 0.3, 1.0 and 3.0 percent. Results obtained from microscopic examination of tissues and organs disclosed microconcretions in the renal tubules of the female rats from all three test groups. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related. No abnormalities were observed in any of the following parameters:

Body Weight Gains
Food Consumption
Hematologic Studies
Clinical Blood Chemistry Studies
Urine Analyses
Gross Pathologic Studies
Organ Weights and Ratios

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Philip S. Smith

Philip S. Smith, B.S.
Assistant Toxicologist
Rat Toxicity

Report approved by:

James B. Plank

James B. Plank
Senior Group Leader
Rat Toxicity

Paul L. Wright

Paul L. Wright, Ph.D.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

slg

STAUFFER CHEMICAL COMPANY
Industrial Chemical Division

CERTIFICATE OF ANALYSIS

LEVAIR,
Material SODIUM ALUMINUM PHOSPHATE, ACIDIC, POWD. Date 9-29-71

Identification SAMPLE REPRESENTING 22 BAGS OF LEVAIR CODED 3-22-DCF-1 PREPARED FOR NINETY-DAY FEEDING STUDIES.

ANALYSIS:		FCC LIMITS
Assay, % $NaAl_3H_{14}(PO_4)_8.4H_2O$	99.9	95 Min.
% LOI	19.5	19.5 - 21.0
% Free Acid	0.2	---
Neutralizing Value	100	100 Min.
Arsenic, PPM As	0.1	3 Max.
Fluoride, PPM F	10	25 Max.
Heavy Metals, PPM (as Pb)	<30	40 Max.
Lead, PPM Pb	0.1	10 Max.
Particle Size		
% On 100	1.5	---
% On 140	5.1	---

THIS MATERIAL COMPLIES WITH FOOD CHEMICALS CODEX SPECIFICATIONS.

R E Benjamin

Sworn and subscribed to before me this
29th day of September, 1971.

Ralph Worthy

My Commission Expires 10-31-71.

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. One hundred and twenty rats (60 males and 60 females) were selected for the experiment and housed individually in standard, wire-bottomed steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, individual animal number and sex.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Outline of Experiment

Group	Number of Animals Males	Females	Dietary Level (percent)
Control	15	15	None Administered
T-I	15	15	0.3
T-II	15	15	1.0
T-III	15	15	3.0

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at weekly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period and the data subjected to statistical analyses.

D. Food Consumption and Diet Preparation

Food consumption data were collected individually for five rats of each sex in every group weekly during the study and the data recorded.

The diet for any given group was prepared by blending the appropriate amount of Levair with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one weeks' ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Hematologic, Clinical Blood Chemistry Studies and Urine Analyses

Blood and urine samples collected individually from ten rats of each sex from both the control and T-III groups after 45 and 84 days of feeding were analyzed for the following:

1. Hematologic Studies

   a. Hematocrit Value
   b. Erythrocyte Count
   c. Hemoglobin Concentration
   d. Total Leukocyte Count
   e. Differential Leukocyte Count

2. Clinical Blood Chemistry Studies

   a. Blood Urea Nitrogen (BUN) Concentration
   b. Serum Alkaline Phosphatase (SAP) Activity
   c. Serum Glutamic-Pyruvic Transaminase (SGPT) Activity
   d. Fasted Blood Glucose Concentration

3. Urine Analyses

   a. Glucose Concentration
   b. Albumin Concentration
   c. Microscopic Elements Examination
   d. pH
   e. Specific Gravity

G. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of gross examination a complete set of organs and other tissues was removed from each rat and preserved in formalin solution. Also at autopsy the weights of the liver, kidneys, spleen, gonads, heart and brain of each rat were determined and recorded.

Microscopic examination of tissues taken from ten rats of each sex from both the control and T-III groups was conducted. The following tissues, stained with Hematoxylin-Eosin, were included: esophagus,

stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate gland, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

H. Organ Weights, Organ to Body Weight and Organ to Brain Weight Ratios

Statistical analyses were conducted upon the absolute organ weights and their corresponding ratios to the weight of the body and brain. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Statistical comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (percent)	Sex	Body Weight (grams) Week: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Weight Gain (grams/rat)
Control	M	185	242	298	349	361	391	417	437	458	481	515	518	521	522	337
	F	150	185	211	232	245	253	260	275	281	291	304	301	318	331	181
0.3	M	187	237	295	339	366	394	412	435	450	474	504	509	520	527	340
	F	149	192	215	234	245	259	271	281	287	294	306	310	311	315	166
1.0	M	186	234	285	334	371	398	424	447	458	487	509	518	533	541	355
	F	148	193	210	233	241	252	262	273	279	288	298	296	303	310	162
3.0	M	185	239	299	359	381	413	436	458	444	500	524	524	529	538	353
	F	149	182	204	227	243	253	267	271	281	288	294	294	307	316	167

B. Food Consumption

Food consumption data collected during the 90-day test period are summarized in Table III.

Test rats ate amounts of food comparable to that consumed by control rats.

TABLE III

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Food Consumption Data

Summary of Mean Values

Dietary Level (percent)	Sex	Food Consumption (grams/rat/seven days) Week: 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption (grams/rat)
Control	M	154	148	169	163	191	204	124	206	185	201	186	191	174	2296
	F	108	117	125	117	147	149	97	148	160	128	117	127	165	1705
0.3	M	153	139	173	169	175	210	124	193	170	191	183	184	174	2238
	F	116	123	119	129	186	153	96	142	125	123	132	124	123	1691
1.0	M	153	141	166	152	189	193	109	187	174	177	191	181	195	2208
	F	132	121	110	116	132	141	68	136	128	133	142	136	150	1645
3.0	M	168	166	186	184	201	201	134	202	195	201	188	209	167	2402
	F	113	120	128	118	138	143	80	130	115	114	105	123	123	1550

C. Mortality and Reactions

Four deaths occurred during the study. All of these deaths resulted from trauma incurred during the collection of blood samples.

No untoward behavioral reactions were noted among any of the animals employed in the study.

D. Hematologic Studies

The results of the hematologic studies conducted on blood samples taken from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables IV and V.

No outstanding differences between test and control rats were noted with respect to any of the parameters investigated.

TABLE IV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Total Leukocyte Count (thousands/mm^3) Day: 45	84	Erythrocyte Count (millions/mm^3) Day: 45	84	Hemoglobin Concentration (g/100 ml) Day: 45	84	Hematocrit Value (%) Day: 45	84
Control	M	17.1	15.9	7.72	8.08	15.7	15.9	39.3	40.5
	F	14.7	9.9	7.70	7.52	15.5	15.7	38.4	37.9
3.0	M	15.0	13.4	7.60	8.05	15.6	15.8	38.8	40.1
	F	13.8	8.6	7.30	7.46	15.2	15.6	37.6	38.3

TABLE V

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Differential Leukocyte Count (Number of Cells per Hundred) Lymphocytes Day: 45	84	Neutrophils Day: 45	84	Monocytes Day: 45	84	Eosinophils Day: 45	84	Basophils Day: 45	84
Control	M	90.6	86.7	8.6	11.1	0.8	1.7	0.0	0.5	0.0	0.0
	F	83.2	85.5	15.2	12.6	0.8	1.0	0.8	0.9	0.0	0.0
3.0	M	81.8	85.8	16.2	11.5	1.4	2.1	0.6	0.6	0.0	0.0
	F	84.8	84.3	13.8	13.6	1.0	1.5	0.4	0.6	0.0	0.0

E. Clinical Blood Chemistry Studies

The results of the clinical chemistry studies conducted on blood samples obtained from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables VI and VII.

Values for test rats were not different from those of control rats.

TABLE VI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Serum Alkaline Phosphatase Activity (King-Armstrong Units) Day: 45	84	Serum Glutamic-Pyruvic Transaminase Activity (Dade Units) Day: 45	84
Control	M	31	24	30	27
	F	20	12	24	28
3.0	M	33	22	25	29
	F	18	19	27	29

TABLE VII

TEST MATERIAL: **Levair**

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Blood Urea Nitrogen Concentration (mgs %) Day: 45	Blood Urea Nitrogen Concentration (mgs %) Day: 84	Fasted Blood Glucose Concentration (mgs %) Day: 45	Fasted Blood Glucose Concentration (mgs %) Day: 84
Control	M	16	15	129	141
	F	17	14	130	137
3.0	M	15	14	146	134
	F	16	13	140	135

F. Urine Analyses

The results of the periodic examinations of urine specimens collected from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Table VIII.

No significant differences between the urine of the test rats and control rats were observed.

TABLE VIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Urine Analysis Data

Summary of Mean Values

Dietary Level (percent)	Sex	Glucose Day: 45	Glucose Day: 84	Albumin Day: 45	Albumin Day: 84	Microscopic Elements Day: 45	Microscopic Elements Day: 84	pH Day: 45	pH Day: 84	Specific Gravity Day: 45	Specific Gravity Day: 84
Control	M	n	n	t	n	+1	+2	7.0	6.8	1.025	1.039
	F	n	n	n	n	+2	+1	7.8	6.8	1.027	1.036
3.0	M	n	n	n	t	+1	t	6.6	6.4	1.031	1.049
	F	n	n	n	t	+2	t	7.2	6.4	1.016	1.030

Glucose and Albumin

n = negative
t = trace; less than 30 mg/100 ml urine
+1 = 30 to 100 mg/100 ml urine
+2 = 100 to 300 mg/100 ml urine
+3 = 300 to 500 mg/100 ml urine
+4 = more than 500 mg/100 ml urine

Microscopic Elements

N = normal
t = minimal or trace amounts
+1 = slight amounts
+2 = moderate amounts
+3 = large amounts
+4 = extreme amounts

G. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Organ Weight and Organ to Body and Organ to Brain Weight Ratio Data

The results of the statistical analyses conducted on absolute organ weights, organ to body weight and organ to brain weight ratios are summarized in Tables IX through XIV.

There were no statistically significant intergroup differences.

TABLE IX

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - LIVER

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	18.140	10.064	3.4790	3.2526	8.8872	5.0896
0.3	19.337	10.426	3.6944	3.3486	9.5023	5.5466
1.0	19.647	10.158	3.6202	3.2744	9.3073	5.2242
3.0	19.629	9.357	3.6515	3.1693	9.3964	4.8503

O STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE X

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.666	2.117	0.7019	0.6798	1.8015	1.0695
0.3	3.699	2.048	0.7104	0.6596	1.8193	1.0902
1.0	3.636	2.157	0.6722	0.6984	1.7239	1.1090
3.0	3.886	2.104	0.7230	0.7153	1.8586	1.0908

STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XI

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - SPLEEN

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	0.936	0.618	0.1805	0.2031	0.4599	0.3087
0.3	0.852	0.557	0.1632	0.1793	0.4184	0.2956
1.0	0.847	0.571	0.1563	0.1851	0.4017	0.2937
3.0	0.937	0.572	0.1740	0.1920	0.4501	0.2949

STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XII

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - GONADS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.611	0.077	0.6924	0.0253	1.7724	0.0392
0.3	3.414	0.079	0.6604	0.0256	1.6797	0.0423
1.0	3.669	0.099	0.6806	0.0321	1.7383	0.0510
3.0	3.580	0.096	0.6672	0.0331	1.7155	0.0498

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XIII

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - HEART

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	1.613	1.078	0.3095	0.3484	0.7937	0.5447
0.3	1.653	1.052	0.3174	0.3383	0.8135	0.5599
1.0	1.754	1.129	0.3232	0.3662	0.8314	0.5842
3.0	1.685	1.054	0.3139	0.3599	0.8094	0.5481

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE XIV

TEST MATERIAL - LEVAIR

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - BRAIN

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	2.041	1.982	0.3914	0.6431	1.0000	1.0000
0.3	2.035	1.881	0.3924	0.6071	1.0000	1.0000
1.0	2.111	1.947	0.3915	0.6317	1.0000	1.0000
3.0	2.089	1.932	0.3894	0.6563	1.0000	1.0000

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

3. Histopathologic Findings

Histopathologic examination of tissues and organs taken from ten rats of each sex in both the control and T-III groups was conducted. Microscopic examination of sections of kidneys taken from ten rats of each sex from the T-II group and from all female rats from the T-I group was also conducted.

Tables XV through XVIII list all histopathologic changes noted.

IBT No. B747
Stauffer Chemical Company

I have completed a histopathologic evaluation of a series of rat tissues from IBT No. B747. There are microconcretions present in the renal tubules of the female rats from all three dose levels. These concretions are located in the tubules at the corticomedullary junction and they consist of an amorphous material which shattered on sectioning. They are blue in color and are probably calcified. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related.

The other lesions observed are those of spontaneous disease and they are not unusual for the albino rat.

Ward R Richter

Ward R. Richter, D.V.M., M.S.
Diplomate, American College of
Veterinary Pathologists

TABLE XV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: Control

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Chronic tracheitis	6	1.0
	Lung	Chronic murine pneumonia	3	1.0
	Colon	Parasites	1	1.0
	Kidney	Focal lymphoid infiltration	3	1.0
	Urinary bladder	Mucoid plug	6	1.0
10 Females	Lung	Chronic murine pneumonia	1	1.0
	Colon	Parasites	1	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-I (0.3%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
15 Females	Kidney	Microconcretions	3	0.5
		Tubular nephrosis	1	1.0
		Chronic nephritis	1	2.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVII

TEST MATERIAL: **Levair**

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-II (1.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Kidney	Tubular nephrosis	1	1.0
10 Females	Kidney	Microconcretions	7	1.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-III (3.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Heart	Chronic focal myocarditis	1	1.0
	Trachea	Chronic tracheitis	8	2.0
	Lung	Chronic murine pneumonia	5	1.0
	Kidney	Focal interstitial nephritis	1	1.0
	Testes	Focal necrosis	1	1.0
10 Females	Trachea	Chronic tracheitis	1	1.0
	Lung	Chronic murine pneumonia	3	1.0
	Kidney	Microconcretions	7	1.0
		Focal interstitial nephritis	2	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

JUL 31 1972

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

NDS 0179

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVAIR IN BEAGLE DOGS

JULY 7, 1972

IBT NO. J749

51

ABL	GDM	HM	RLR	JS
JUL 11 1972				
RECEIVED, RICHMOND				

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

July 7, 1972

Mr. A. B. Lindquist, Manager
Product Registrations
Stauffer Chemical Company
1200 South 47th Street
Richmond, Claifornia 98404

Dear Mr. Lindquist:

Re: IBT No. J749 - 90/Day Subacute Oral Toxicity Study with Levair in Beagle Dogs

We are submitting herewith our laboratory report dated July 7, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/lk

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH
LEVAIR
IN BEAGLE DOGS

JULY 7, 1972

IBT NO. J749

I. Introduction

A sample identified as Levair was received from Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using purebred beagle dogs. The following report presents the results of the investigation.

II. Summary

Ninety-day oral administration of Levair to purebred beagle dogs at dietary levels of 0.3, 1.0 and 3.0 percent revealed no significant abnormalities in the following parameters:

Body Weights	Hematologic Studies
Food Consumption	Blood Chemistry Studies
Behavioral Reactions	Urine Analyses
Mortality	Gross Pathologic Studies
	Histopathologic Studies

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Kenneth Mastalski, B.S.
Group Leader
Wedge's Creek Research Farm

Report approved by:

Donald H. Jenkins, D.V.M.
Manager & Technical Director
Wedge's Creek Research Farm

M. L. Keplinger, Ph.D.
Manager, Toxicology

bb

CERTIFICATE OF ANALYSIS

LEVAIR,
Material SODIUM ALUMINUM PHOSPHATE, ACIDIC, POWD. Date 9-29-71

Identification SAMPLE REPRESENTING 22 BAGS OF LEVAIR CODED 3-22-DCF-9 PREPARED FOR NINETY-DAY FEEDING STUDIES.

ANALYSIS:		FCC LIMITS
Assay, % $NaAl_3H_{14}(PO_4)_8.4H_2O$	99.9	95 Min.
% LOI	19.5	19.5 - 21.0
% Free Acid	0.2	---
Neutralizing Value	100	100 Min.
Arsenic, PPM As	0.1	3 Max.
Fluoride, PPM F	10	25 Max.
Heavy Metals, PPM (as Pb)	<30	40 Max.
, PPM Pb	0.1	10 Max.
Particle Size		
% On 100	1.5	---
% On 140	5.1	---

THIS MATERIAL COMPLIES WITH FOOD CHEMICALS CODEX SPECIFICATIONS.

R E Benjamin

Sworn and subscribed to before me this 29th day of September, 1971.

Ralph Worthy

My Commission Expires 10-31-71.

III. Procedure

A. Organization

The 90-day toxicity study utilized an untreated control group and three test groups, each consisting of eight purebred beagle dogs (four males and four females). The beagles were all eligible for A.K.C. registration and had been previously immunized against rabies, distemper, infectious canine hepatitis and leptospirosis.

All dogs were acquired from our own colony and were under observation for two weeks prior to the start of the investigation, during which time they were reimmunized and rendered clinically free of any existing parasitic infestation.

During the investigation, the selected animals were housed in kennels equipped with outside runs, four dogs of the same sex and group being accommodated in a single kennel.

The material to be tested, Levair, was incorporated into a stock diet and fed to the dogs seven days a week at three graded dietary levels. The levels were 0.3, 1.0 and 3.0 percent of test material in the diet.

An outline of the test organization and diet composition is presented in Table I. A certificate of test material analysis is also presented.

TABLE I

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Test Organization and Diet Composition

Group	Number of Animals		Constituents of Diet	
	Male	Female	Dose Level (%)	Stock Ration* (%)
UC	4	4	None	100.0
T-I	4	4	0.3	99.7
T-II	4	4	1.0	99.0
T-III	4	4	3.0	97.0

* Golden Choice Meals, Adolph Coors Company, Denver, Colorado.

B. Parameters Investigated

Initially, the body weight of each dog in every group was determined and recorded. Thereafter, weighings were conducted weekly for the duration of the test.

At the beginning of each week, the appropriate dietary constituents for each of the groups were thoroughly blended in a Hobart mixer. Preweighed amounts were distributed into self-feeding units and maintained in excess of the animals' consumption. One such unit was available to the dogs in each kennel on an ad libitum basis 24 hours a day. At the end of each seven-day period, all unconsumed food was collected and weighed. Food consumption was then calculated and recorded. Water was available to the animals at all times.

The dogs were under observation during the investigation and were examined daily for clinical signs or symptoms indicative of systemic toxicity.

The following determinations were conducted upon each dog from the untreated control group and three test groups just prior to the inception of the study and after 42 and 84 days of testing:

Hematologic Studies

total leukocyte count
erythrocyte count
hemoglobin
hematocrit
differential leukocyte count

Blood Chemistry Studies

blood urea nitrogen
serum glucose
serum alkaline phosphatase
serum glutamic-oxalacetic transaminase
serum glutamic-pyruvic transaminase

Urine Analyses

albumin
glucose
pH
microscopic elements - leukocytes
erythrocytes
crystals

At the conclusion of the investigation, the dogs from each group were sacrificed by electric shock. All major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland and pituitary gland. The following tissues and organs excised from these animals were examined histologically (Hematoxylin-Eosin Stain):

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lungs
Lymph Nodes (cervical, mesenteric)
Muscle (skeletal)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (submaxillary)
Small Intestine (duodenum, jejunum, ileum)
Spinal Cord
Spleen
Stomach (cardia, fundus, pylorus)
Trachea
Thyroid Gland
Uterus
Urinary Bladder

IV. Results

A. Body Weight Data

The body weight data are presented in Tables II and III.

No significant deviations from normally expected body weight gains for dogs of this age were noted.

TABLE II

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Males, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	1	5.5	10.0	10.1	10.1	9.8	10.3	10.0	11.0	10.8	10.6	10.8	11.1	11.1	10.9	11.0	1.0
		2	6.0	9.5	9.7	9.7	10.3	10.5	10.4	11.1	11.3	11.4	11.7	12.1	12.1	12.2	11.9	2.4
		3	6.0	10.2	10.1	10.5	10.8	11.2	11.6	11.5	11.8	11.7	12.2	12.5	12.3	11.9	12.3	2.1
		4	6.0	8.4	8.5	8.6	8.6	8.9	8.5	9.3	9.5	9.5	9.8	10.2	10.0	9.9	10.2	1.8
		Mean	5.9	9.5	9.6	9.7	9.9	10.2	10.1	10.7	10.8	10.8	11.1	11.5	11.4	11.2	11.4	1.9
T-I	0.3	9	5.5	10.2	10.4	11.0	11.4	11.6	11.6	11.8	12.0	12.1	12.5	12.3	12.5	12.2	12.5	2.3
		10	5.5	9.8	10.0	10.4	10.6	10.7	10.7	10.9	11.2	11.1	11.3	11.4	11.5	11.1	11.8	2.0
		11	5.5	8.7	9.0	9.2	9.3	9.8	9.8	9.7	10.0	9.7	10.5	10.6	10.7	10.3	11.0	2.3
		12	6.0	8.4	8.6	9.2	9.1	9.5	9.8	10.1	10.1	10.3	10.6	10.7	11.0	10.6	11.0	2.6
		Mean	5.6	9.3	9.5	10.0	10.1	10.4	10.5	10.6	10.8	10.8	11.2	11.2	11.4	11.0	11.6	2.3
T-II	1.0	17	5.5	9.3	9.3	9.5	9.9	10.2	10.2	10.1	10.4	10.5	10.5	10.5	10.6	10.5	10.4	1.1
		18	5.5	8.8	9.1	9.5	10.1	10.0	10.4	10.7	10.8	10.7	10.8	11.1	11.2	10.9	11.3	2.5
		19	5.8	8.7	8.8	9.0	9.2	9.3	9.5	9.6	10.0	9.8	10.2	10.1	10.1	10.1	10.6	1.9
		20	6.0	8.3	8.5	9.0	9.5	9.7	10.0	10.4	10.6	10.8	11.1	11.1	11.4	11.2	11.5	3.2
		Mean	5.7	8.8	8.9	9.2	9.7	9.8	10.0	10.2	10.4	10.4	10.6	10.7	10.8	10.7	11.0	2.2
T-III	3.0	25	5.5	8.7	9.2	9.7	9.6	10.2	10.1	10.3	10.7	10.7	10.9	10.8	11.2	10.8	11.2	2.5
		26	5.8	8.2	8.2	8.3	8.7	9.1	9.1	9.3	9.5	9.3	9.6	9.6	9.8	9.6	9.7	1.5
		27	6.0	9.0	9.5	10.1	10.5	11.1	11.1	11.7	11.7	11.8	12.0	12.3	12.6	12.4	12.6	3.6
		28	6.0	6.7	6.9	7.1	7.6	8.1	7.8	8.5	8.6	8.5	8.8	8.7	8.9	8.8	9.2	2.5
		Mean	5.8	8.2	8.4	8.8	9.1	9.6	9.5	10.0	10.1	10.1	10.3	10.4	10.6	10.4	10.7	2.5

TABLE III

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Females, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	5	6.0	6.6	6.9	7.1	7.3	7.3	7.4	7.8	7.8	7.8	8.0	8.1	8.2	8.0	8.1	1.5
		6	6.0	7.9	8.1	8.4	8.3	8.4	8.1	9.1	8.9	8.9	9.1	9.1	9.3	9.2	9.2	1.3
		7	6.0	8.0	8.4	8.8	8.7	9.0	9.0	9.7	10.0	9.7	10.2	10.1	10.6	10.5	10.5	2.5
		8	6.0	6.7	6.6	6.9	7.1	7.4	7.1	7.7	7.8	7.8	8.0	8.2	8.2	8.0	8.0	1.3
		Mean	6.0	7.3	7.5	7.8	7.8	8.0	7.9	8.6	8.6	8.6	8.8	8.9	9.1	8.9	9.0	1.7
T-I	0.3	13	5.5	7.2	6.9	6.9	7.1	7.4	7.5	7.4	7.9	7.7	8.0	8.1	8.5	8.0	8.2	1.0
		14	5.5	6.9	7.3	7.4	7.6	8.0	8.1	8.3	8.3	8.2	8.5	8.4	8.6	8.3	8.6	1.7
		15	6.0	7.7	5.8	6.1	6.4	6.7	6.5	7.0	7.3	7.3	7.4	7.5	7.3	7.4	7.5	-0.2
		16	6.0	8.2	8.4	8.8	9.2	9.4	9.2	9.5	9.8	9.7	9.9	10.0	10.4	10.0	10.1	1.9
		Mean	5.8	7.5	7.1	7.3	7.6	7.9	7.8	8.0	8.3	8.2	8.4	8.5	8.7	8.4	8.6	1.1
T-II	1.0	21	5.5	7.5	7.7	7.7	8.0	8.0	8.1	8.1	8.4	8.3	8.3	8.3	8.3	8.1	8.6	1.0
		22	5.5	7.1	7.2	7.5	7.6	8.0	7.4	7.9	8.0	8.1	8.1	8.2	8.1	7.8	8.4	1.3
		23	5.5	4.5	4.8	5.0	5.2	5.3	5.3	5.5	5.5	5.6	5.7	5.7	5.6	5.2	5.7	1.2
		24	6.0	6.3	6.5	6.8	7.5	7.5	7.7	7.6	8.0	8.0	8.1	8.6	8.4	9.0	8.6	2.3
		Mean	5.6	6.4	6.6	6.8	7.1	7.2	7.1	7.3	7.5	7.5	7.6	7.7	7.6	7.5	7.8	1.4
T-III	3.0	29	5.5	5.3	5.4	5.9	5.7	6.0	6.0	6.0	6.3	6.3	6.3	6.4	6.4	6.3	6.5	1.2
		30	5.5	5.9	5.9	5.8	6.2	6.4	6.5	6.7	6.9	6.9	7.1	7.2	7.3	6.7	7.3	1.4
		31	6.0	7.1	7.1	7.3	7.7	7.9	8.1	8.1	8.4	8.2	8.4	8.4	8.6	8.3	8.8	1.8
		32	6.0	7.5	7.8	7.8	8.0	8.5	8.7	8.9	8.9	9.2	9.3	9.3	9.6	9.2	9.6	2.1
		Mean	5.8	6.4	6.6	6.7	6.9	7.2	7.3	7.4	7.6	7.6	7.8	7.8	8.0	7.6	8.0	1.6

B. Food Consumption

No significant intergroup differences were noted with respect to the food consumption data.

The data are presented in Table IV.

TABLE IV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Food Consumption Data

	Mean Food Consumed During Week Indicated (g/kg of body weight)							
Sex:	Males				Females			
Group:	UC	T-I	T-II	T-III	UC	T-I	T-II	T-III
Week Dietary Level (%):	None	0.3	1.0	3.0	None	0.3	1.0	3.0
1	352	355	376	428	380	408	417	352
2	361	402	380	398	417	428	408	384
3	366	339	353	382	397	372	380	348
4	338	336	334	398	366	380	403	389
5	356	350	300	325	399	343	339	339
6	348	283	335	420	375	278	376	481
7	319	352	305	384	343	277	367	431
8	285	306	300	309	323	335	323	299
9	315	337	309	335	321	348	332	317
10	333	335	322	336	362	350	282	285
11	300	309	303	325	341	325	319	319
12	286	280	297	315	298	286	333	312
13	281	364	316	316	285	346	378	324
Mean	326	334	325	359	354	344	358	352

C. Reactions

No untoward behavioral reactions were recorded during the investigation.

D. Mortality

No fatalities occurred during the investigation.

E. Hematologic Studies

The results of these determinations are presented in Tables V through IX.

No significant abnormalities were noted at any levels tested.

TABLE V

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Total Leukocyte Count, thousands/mm^3

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	14.2	12.3	9.3
		2-M	19.9	17.2	14.7
		3-M	17.0	18.0	15.4
		4-M	16.4	15.2	10.7
		5-F	12.9	14.6	11.1
		6-F	12.2	14.0	11.9
		7-F	14.0	15.2	11.9
		8-F	9.9	12.7	12.7
T-I	0.3	9-M	19.3	17.0	14.9
		10-M	20.5	17.5	13.4
		11-M	16.8	14.6	12.2
		12-M	25.8	15.2	13.8
		13-F	16.9	14.4	11.7
		14-F	13.8	15.3	11.3
		15-F	16.5	14.8	17.3
		16-F	18.2	13.2	12.9
T-II	1.0	17-M	17.0	15.1	15.2
		18-M	13.4	16.7	12.7
		19-M	12.5	17.3	10.6
		20-M	11.6	14.4	12.0
		21-F	14.9	13.3	11.9
		22-F	15.5	13.1	11.7
		23-F	23.1	17.5	15.4
		24-F	12.8	12.6	11.3
T-III	3.0	25-M	10.8	13.5	11.0
		26-M	16.4	12.9	10.4
		27-M	15.4	15.2	11.8
		28-M	16.9	17.1	13.2
		29-F	12.2	13.8	13.6
		30-F	15.4	15.4	14.3
		31-F	21.8	15.9	12.5
		32-F	13.2	11.6	11.9

TABLE VI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Erythrocyte Count, millions/mm^3

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	6.57	6.64	7.56
		2-M	7.06	6.60	6.89
		3-M	6.45	6.69	6.79
		4-M	5.68	6.06	6.37
		5-F	6.58	6.57	7.09
		6-F	6.50	6.98	6.92
		7-F	6.48	7.10	6.98
		8-F	5.89	6.48	6.76
T-I	0.3	9-M	6.64	6.74	7.57
		10-M	6.65	6.65	7.34
		11-M	6.11	5.88	6.67
		12-M	6.13	6.03	6.87
		13-F	6.38	6.33	7.13
		14-F	6.75	6.34	6.28
		15-F	6.11	6.10	7.18
		16-F	6.12	6.21	7.10
T-II	1.0	17-M	6.55	6.31	7.04
		18-M	6.41	5.90	6.46
		19-M	6.09	6.01	6.75
		20-M	5.53	5.77	6.63
		21-F	6.14	6.31	6.38
		22-F	6.60	6.36	6.94
		23-F	5.93	5.75	6.44
		24-F	5.92	5.86	6.54
T-III	3.0	25-M	6.09	5.77	6.45
		26-M	6.39	5.91	6.68
		27-M	5.91	6.02	6.63
		28-M	5.68	5.45	6.08
		29-F	6.32	6.27	6.75
		30-F	6.26	6.24	7.00
		31-F	6.27	6.64	7.05
		32-F	6.57	6.35	7.02

TABLE VII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Hemoglobin, gm/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15.4	15.5	16.8
		2-M	16.3	15.7	16.0
		3-M	15.5	16.6	16.3
		4-M	14.2	14.8	15.7
		5-F	14.5	15.4	16.6
		6-F	15.3	16.8	16.1
		7-F	15.2	17.3	16.6
		8-F	14.1	15.7	16.1
T-I	0.3	9-M	15.3	15.9	17.8
		10-M	15.1	14.9	16.9
		11-M	13.5	13.3	14.7
		12-M	14.1	13.9	15.8
		13-F	14.0	14.2	15.9
		14-F	15.3	15.2	15.1
		15-F	14.3	14.9	17.2
		16-F	14.4	15.3	17.4
T-II	1.0	17-M	15.0	14.4	16.0
		18-M	14.8	14.0	15.1
		19-M	14.2	14.0	15.8
		20-M	12.9	13.0	15.2
		21-F	14.8	15.3	15.5
		22-F	15.4	14.6	16.5
		23-F	14.5	14.2	15.5
		24-F	12.9	12.9	14.6
T-III	3.0	25-M	14.2	13.7	15.2
		26-M	14.2	13.9	15.6
		27-M	13.2	13.7	15.5
		28-M	13.6	13.7	14.9
		29-F	14.6	15.2	16.4
		30-F	15.5	16.1	17.7
		31-F	13.9	15.3	16.3
		32-F	15.9	15.6	17.6

TABLE VIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Hematocrit, percent

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	43.7	46.4	46.3
		2-M	47.0	45.7	43.2
		3-M	44.9	48.5	44.4
		4-M	39.0	43.7	41.8
		5-F	41.0	44.5	44.4
		6-F	43.0	48.9	44.4
		7-F	43.3	50.4	44.3
		8-F	40.2	46.2	44.8
T-I	0.3	9-M	43.8	46.9	48.4
		10-M	43.3	45.6	45.7
		11-M	38.4	38.9	40.1
		12-M	40.2	41.0	42.9
		13-F	39.2	42.5	43.2
		14-F	44.6	44.6	39.8
		15-F	40.8	43.4	47.1
		16-F	41.4	44.4	47.2
T-II	1.0	17-M	42.8	43.6	44.7
		18-M	42.7	41.5	41.9
		19-M	41.4	42.1	43.7
		20-M	35.8	39.0	41.3
		21-F	42.3	46.2	43.1
		22-F	42.8	43.8	44.3
		23-F	40.8	42.0	43.0
		24-F	37.1	38.0	39.5
T-III	3.0	25-M	39.7	40.9	41.9
		26-M	40.6	40.5	42.5
		27-M	38.2	40.8	42.5
		28-M	39.0	40.1	41.6
		29-F	42.6	45.9	45.0
		30-F	43.9	47.0	47.9
		31-F	40.2	45.8	44.7
		32-F	45.7	47.1	47.8

TABLE IX

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Neutrophils

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	66	74	56
		2-M	77	70	69
		3-M	49	66	57
		4-M	56	70	68
		5-F	65	59	66
		6-F	73	59	73
		7-F	46	61	63
		8-F	31	58	68
T-I	0.3	9-M	42	52	62
		10-M	75	77	69
		11-M	70	55	51
		12-M	70	32	37
		13-F	66	62	62
		14-F	56	72	74
		15-F	57	44	66
		16-F	58	57	67
T-II	1.0	17-M	58	55	54
		18-M	48	71	71
		19-M	58	75	62
		20-M	42	65	51
		21-F	51	59	55
		22-F	52	58	59
		23-F	53	65	55
		24-F	57	57	44
T-III	3.0	25-M	50	69	44
		26-M	56	62	56
		27-M	45	59	46
		28-M	54	74	58
		29-F	46	64	62
		30-F	45	67	55
		31-F	66	70	69
		32-F	57	56	56

TABLE IX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Lymphocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	28	19	36
		2-M	17	21	28
		3-M	39	25	33
		4-M	39	21	26
		5-F	26	31	29
		6-F	17	29	25
		7-F	45	27	30
		8-F	64	38	21
T-I	0.3	9-M	50	39	30
		10-M	21	20	28
		11-M	26	39	46
		12-M	24	55	46
		13-F	29	35	33
		14-F	36	21	20
		15-F	36	43	21
		16-F	39	36	25
T-II	1.0	17-M	35	34	33
		18-M	48	24	26
		19-M	31	16	33
		20-M	47	28	42
		21-F	38	36	40
		22-F	42	35	36
		23-F	39	22	39
		24-F	35	34	47
T-III	3.0	25-M	44	28	44
		26-M	38	27	33
		27-M	50	31	48
		28-M	39	24	35
		29-F	51	30	30
		30-F	48	26	40
		31-F	27	23	30
		32-F	36	35	39

TABLE IX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Monocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	2	4	3
		2-M	3	1	1
		3-M	2	2	0
		4-M	0	4	1
		5-F	0	3	1
		6-F	2	5	0
		7-F	3	5	0
		8-F	0	0	2
T-I	0.3	9-M	4	4	2
		10-M	3	0	1
		11-M	2	2	1
		12-M	3	5	3
		13-F	1	3	2
		14-F	4	1	3
		15-F	1	1	3
		16-F	1	1	2
T-II	1.0	17-M	1	6	5
		18-M	3	1	1
		19-M	3	3	0
		20-M	2	1	4
		21-F	6	1	2
		22-F	0	1	1
		23-F	4	3	4
		24-F	4	0	1
T-III	3.0	25-M	2	0	1
		26-M	3	3	0
		27-M	3	1	3
		28-M	2	0	1
		29-F	0	1	2
		30-F	1	4	3
		31-F	3	1	1
		32-F	3	2	0

TABLE IX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Eosinophils

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	4	3	5
		2-M	3	8	2
		3-M	10	7	10
		4-M	5	5	5
		5-F	9	7	4
		6-F	8	7	2
		7-F	6	7	7
		8-F	5	4	9
T-I	0.3	9-M	4	5	6
		10-M	1	3	2
		11-M	2	4	2
		12-M	3	8	14
		13-F	4	0	3
		14-F	4	6	3
		15-F	6	12	10
		16-F	2	6	6
T-II	1.0	17-M	6	5	8
		18-M	1	4	2
		19-M	8	6	5
		20-M	9	6	3
		21-F	5	4	3
		22-F	6	6	4
		23-F	4	10	2
		24-F	4	9	8
T-III	3.0	25-M	4	3	11
		26-M	3	8	11
		27-M	2	9	3
		28-M	5	2	6
		29-F	3	5	6
		30-F	6	3	2
		31-F	4	6	0
		32-F	4	6	0

TABLE IX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Basophils

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	0
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

F. Blood Chemistry Studies

The results of these determinations are presented in Tables X through XIV.

There is no significant difference between the untreated control and the three test groups.

TABLE X

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Blood Urea Nitrogen, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	20	20	14
		2-M	13	15	12
		3-M	16	20	14
		4-M	16	23	12
		5-F	15	19	11
		6-F	10	15	11
		7-F	11	12	10
		8-F	16	19	12
T-I	0.3	9-M	12	13	12
		10-M	11	13	12
		11-M	14	16	16
		12-M	14	17	14
		13-F	22	19	15
		14-F	21	16	15
		15-F	17	16	16
		16-F	22	13	10
T-II	1.0	17-M	12	10	8
		18-M	12	13	9
		19-M	13	15	12
		20-M	15	21	17
		21-F	13	15	11
		22-F	16	13	9
		23-F	16	17	10
		24-F	16	17	11
T-III	3.0	25-M	13	14	10
		26-M	11	13	9
		27-M	15	12	10
		28-M	14	12	9
		29-F	13	14	12
		30-F	18	13	11
		31-F	16	17	9
		32-F	15	15	10

Note: Colorimetric method by Roeckelt and Cochman.

TABLE XI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glucose, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	90	112	105
		2-M	127	125	108
		3-M	118	125	113
		4-M	115	103	97
		5-F	104	113	104
		6-F	121	123	99
		7-F	97	103	102
		8-F	83	108	96
T-I	0.3	9-M	118	93	94
		10-M	125	103	101
		11-M	107	86	87
		12-M	118	97	87
		13-F	101	95	89
		14-F	106	93	94
		15-F	92	97	87
		16-F	112	92	87
T-II	1.0	17-M	127	117	101
		18-M	111	103	96
		19-M	104	106	97
		20-M	109	103	94
		21-F	118	103	99
		22-F	120	105	96
		23-F	127	100	94
		24-F	132	112	99
T-III	3.0	25-M	115	109	97
		26-M	120	103	94
		27-M	132	109	110
		28-M	115	103	96
		29-F	107	101	91
		30-F	125	120	121
		31-F	112	113	97
		32-F	135	112	113

Note: Colorimetric method by Roeckelt and Gochman.

TABLE XII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Alkaline Phosphatase, King-Armstrong Units

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7.06	6.63	5.23
		2-M	6.87	6.34	5.23
		3-M	6.68	6.78	5.06
		4-M	17.33	25.62	13.82
		5-F	9.45	9.10	7.36
		6-F	7.83	8.14	6.81
		7-F	7.44	7.52	6.10
		8-F	4.51	5.23	3.74
T-I	0.3	9-M	5.75	5.50	4.56
		10-M	7.44	9.10	6.81
		11-M	5.75	6.06	5.06
		12-M	6.68	5.23	5.75
		13-F	5.57	5.50	4.89
		14-F	6.30	6.06	4.73
		15-F	12.29	10.12	10.71
		16-F	7.83	7.83	6.28
T-II	1.0	17-M	5.57	5.64	4.73
		18-M	5.94	6.34	8.88
		19-M	10.73	14.14	17.35
		20-M	7.64	7.83	8.69
		21-F	8.23	10.12	8.30
		22-F	7.83	6.63	5.58
		23-F	7.83	7.22	5.75
		24-F	9.87	11.00	11.56
T-III	3.0	25-M	7.25	6.63	6.28
		26-M	8.63	6.92	6.28
		27-M	7.64	6.34	5.92
		28-M	11.39	12.51	10.71
		29-F	12.29	12.31	10.71
		30-F	7.83	6.63	5.40
		31-F	9.45	8.94	7.36
		32-F	9.66	10.64	11.13

Note: Colorimetric method by Babson.

TABLE XIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Oxalacetic Transaminase, Dade Units

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	17	16	18
		2-M	24	21	21
		3-M	24	17	19
		4-M	39	20	27
		5-F	32	20	20
		6-F	14	10	18
		7-F	27	22	15
		8-F	14	15	14
T-I	0.3	9-M	17	28	12
		10-M	27	26	28
		11-M	22	19	16
		12-M	9	22	15
		13-F	21	26	22
		14-F	18	23	19
		15-F	17	24	19
		16-F	13	23	20
T-II	1.0	17-M	13	24	19
		18-M	17	26	14
		19-M	34	32	23
		20-M	24	30	19
		21-F	13	26	21
		22-F	23	22	19
		23-F	22	30	22
		24-F	26	29	16
T-III	3.0	25-M	24	24	24
		26-M	23	26	20
		27-M	35	30	25
		28-M	39	31	28
		29-F	36	30	28
		30-F	35	32	29
		31-F	36	35	25
		32-F	28	28	19

Note: Fluorometric method by Levine and Hill.

TABLE XIV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Pyruvic Transaminase, Dade Units

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15	15	17
		2-M	66	19	27
		3-M	15	16	24
		4-M	15	15	27
		5-F	15	14	15
		6-F	15	15	16
		7-F	15	15	16
		8-F	15	15	18
T-I	0.3	9-M	15	16	17
		10-M	15	18	27
		11-M	16	20	26
		12-M	15	17	22
		13-F	15	17	16
		14-F	14	15	16
		15-F	15	17	24
		16-F	15	15	15
T-II	1.0	17-M	15	23	27
		18-M	15	19	30
		19-M	22	27	32
		20-M	25	28	25
		21-F	18	25	31
		22-F	19	21	26
		23-F	15	18	22
		24-F	27	28	18
T-III	3.0	25-M	21	25	29
		26-M	15	15	19
		27-M	16	20	30
		28-M	15	26	31
		29-F	15	15	35
		30-F	15	19	27
		31-F	15	28	22
		32-F	18	23	35

Note: Fluorometric method by Levine and Hill

G. Urine Analyses

The results of the urine analyses are presented in Tables XV through XVIII.

Urinalysis revealed no significant abnormalities at any of the levels tested.

TABLE XV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Albumin, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	10	0	0
		2-M	10	0	0
		3-M	0	0	0
		4-M	10	10	0
		5-F	0	0	0
		6-F	10	11-50	0
		7-F	10	0	11-50
		8-F	0	0	0
T-I	0.3	9-M	10	0	0
		10-M	10	0	0
		11-M	10	0	0
		12-M	10	0	0
		13-F	11-50	11-50	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	10	0	0
		18-M	0	0	0
		19-M	10	0	0
		20-M	0	0	10
		21-F	0	11-50	11-50
		22-F	0	10	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	10
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

Note: BUMINTEST, Ames Company, Inc., Elkhart, Indiana.

TABLE XVI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Glucose, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	0
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

Note: COMBISTIX, Ames Company, Inc., Elkhart, Indiana.

TABLE XVII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: pH

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7	6	7
		2-M	8	7	6
		3-M	7	7	5
		4-M	7	6	7
		5-F	6	8	8
		6-F	8	7	7
		7-F	8	7	6
		8-F	6	8	7
T-I	0.3	9-M	6	6	6
		10-M	6	7	6
		11-M	8	6	6
		12-M	6	7	7
		13-F	6	7	7
		14-F	6	7	6
		15-F	6	7	6
		16-F	6	6	8
T-II	1.0	17-M	6	8	8
		18-M	6	7	6
		19-M	7	6	6
		20-M	7	6	5
		21-F	6	7	6
		22-F	6	7	5
		23-F	6	7	6
		24-F	7	8	8
T-III	3.0	25-M	6	5	5
		26-M	6	7	5
		27-M	6	7	5
		28-M	6	6	5
		29-F	8	8	7
		30-F	7	7	6
		31-F	7	6	6
		32-F	6	6	6

Note: COMBISTIX, Ames Company, Inc., Elkhart, Indiana.

TABLE XVIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements, average per high power field - Leukocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	20	7	0
		8-F	3	0	0
T-I	0.3	9-M	0	0	0
		10-M	12	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	3	0
		15-F	4	4	5
		16-F	0	0	15
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	6	0	25
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	8
		30-F	6	0	0
		31-F	4	0	0
		32-F	0	0	0

TABLE XVIII continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements, average per high power field - Erythrocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	25*
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	5	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

* in estrus.

TABLE XVIII continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements, average per high power field - Crystals

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	1-3	1-3	1-3
		2-M	1-3	1-3	1-3
		3-M	1-3	1-3	1-3
		4-M	1-3	1-3	1-3
		5-F	1-3	1-3	1-3
		6-F	1-3	1-3	1-3
		7-F	1-3	1-3	1-3
		8-F	1-3	1-3	1-3
T-I	0.3	9-M	1-3	1-3	1-3
		10-M	1-3	1-3	1-3
		11-M	4	1-3	1-3
		12-M	4	1-3	4
		13-F	1-3	1-3	5-8
		14-F	1-3	1-3	1-3
		15-F	1-3	1-3	1-3
		16-F	1-3	1-3	4
T-II	1.0	17-M	1-3	1-3	1-3
		18-M	1-3	1-3	1-3
		19-M	1-3	1-3	1-3
		20-M	4	1-3	1-3
		21-F	1-3	1-3	1-3
		22-F	4	1-3	1-3
		23-F	1-3	1-3	1-3
		24-F	1-3	1-3	1-3
T-III	3.0	25-M	1-3	1-3	1-3
		26-M	1-3	1-3	1-3
		27-M	1-3	1-3	4
		28-M	1-3	1-3	1-3
		29-F	1-3	1-3	1-3
		30-F	1-3	1-3	1-3
		31-F	1-3	1-3	1-3
		32-F	1-3	1-3	1-3

H. Pathologic Findings

1. Organ Weight Data

The organ weight data are presented in Tables XIX through XXVII.

No significant abnormalities were noted among any levels tested.

TABLE XIX

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Liver

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	400.0	36.7
		2-M	402.1	34.7
		3-M	445.9	37.5
		4-M	364.4	36.8
		5-F	273.9	34.2
		6-F	301.7	33.9
		7-F	439.2	42.2
		8-F	294.2	37.7
T-I	0.3	9-M	477.0	38.5
		10-M	391.7	33.5
		11-M	451.0	41.0
		12-M	371.8	33.8
		13-F	258.8	31.6
		14-F	367.3	42.7
		15-F	266.8	35.1
		16-F	335.0	33.2
T-II	1.0	17-M	335.0	32.8
		18-M	378.4	34.7
		19-M	374.7	36.4
		20-M	354.8	31.4
		21-F	328.3	38.6
		22-F	354.1	42.7
		23-F	248.6	44.4
		24-F	231.2	27.2
T-III	3.0	25-M	351.0	32.2
		26-M	295.0	31.0
		27-M	358.2	29.1
		28-M	310.3	34.5
		29-F	229.0	35.8
		30-F	228.9	32.7
		31-F	288.4	33.9
		32-F	274.7	28.9

TABLE XX

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Kidneys

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	68.7	6.30
		2-M	69.7	6.01
		3-M	68.4	5.75
		4-M	71.4	7.21
		5-F	48.5	6.06
		6-F	57.2	6.43
		7-F	64.8	6.23
		8-F	48.4	6.20
T-I	0.3	9-M	77.3	6.23
		10-M	79.2	6.77
		11-M	63.1	5.74
		12-M	75.7	6.88
		13-F	41.7	5.08
		14-F	50.5	5.87
		15-F	46.1	6.07
		16-F	57.6	5.70
T-II	1.0	17-M	74.0	7.26
		18-M	61.2	5.62
		19-M	62.4	6.06
		20-M	62.5	5.53
		21-F	44.4	5.22
		22-F	50.2	6.05
		23-F	36.1	6.45
		24-F	47.2	5.55
T-III	3.0	25-M	65.0	5.93
		26-M	60.5	6.37
		27-M	75.6	6.15
		28-M	65.2	7.24
		29-F	41.0	6.41
		30-F	39.0	5.57
		31-F	53.0	6.24
		32-F	53.3	5.61

TABLE XXI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Heart

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	91.9	8.43
		2-M	99.3	8.56
		3-M	107.6	9.04
		4-M	86.1	8.70
		5-F	74.3	9.29
		6-F	92.7	10.4
		7-F	78.3	7.53
		8-F	70.9	9.09
T-I	0.3	9-M	101.3	8.17
		10-M	92.7	7.92
		11-M	88.0	8.00
		12-M	94.2	8.56
		13-F	65.7	8.01
		14-F	66.3	7.71
		15-F	66.5	8.75
		16-F	79.6	7.88
T-II	1.0	17-M	94.0	9.22
		18-M	97.2	8.92
		19-M	92.0	8.93
		20-M	91.6	8.11
		21-F	84.2	9.91
		22-F	77.3	9.31
		23-F	56.2	10.0
		24-F	68.7	8.08
T-III	3.0	25-M	95.1	8.72
		26-M	92.6	9.75
		27-M	74.5	6.06
		28-M	86.3	9.59
		29-F	71.2	11.1
		30-F	59.8	8.54
		31-F	81.2	9.55
		32-F	83.6	8.80

TABLE XXII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Brain

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	76.7	7.04
		2-M	86.5	7.46
		3-M	90.5	7.60
		4-M	78.6	7.94
		5-F	82.0	10.2
		6-F	84.4	9.48
		7-F	78.0	7.50
		8-F	78.3	10.0
T-I	0.3	9-M	90.6	7.31
		10-M	83.3	7.12
		11-M	85.4	7.76
		12-M	78.0	7.09
		13-F	75.7	9.23
		14-F	76.8	8.93
		15-F	73.2	9.63
		16-F	84.5	8.37
T-II	1.0	17-M	76.5	7.50
		18-M	87.0	7.98
		19-M	88.0	8.54
		20-M	75.0	6.64
		21-F	71.3	8.39
		22-F	78.3	9.43
		23-F	66.0	11.8
		24-F	74.6	8.78
T-III	3.0	25-M	84.3	7.73
		26-M	74.7	7.83
		27-M	72.0	5.85
		28-M	76.4	8.49
		29-F	68.4	10.7
		30-F	68.0	9.71
		31-F	75.6	8.89
		32-F	74.5	7.84

TABLE XXIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Spleen

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	22.2	2.04
		2-M	16.2	1.40
		3-M	35.8	3.01
		4-M	16.2	1.64
		5-F	16.9	2.11
		6-F	23.6	2.65
		7-F	25.4	2.44
		8-F	18.5	2.37
T-I	0.3	9-M	26.8	2.16
		10-M	19.5	1.67
		11-M	25.8	2.34
		12-M	32.3	2.94
		13-F	16.4	2.00
		14-F	19.2	2.23
		15-F	19.3	2.54
		16-F	21.1	2.09
T-II	1.0	17-M	29.5	2.89
		18-M	24.5	2.25
		19-M	23.0	2.23
		20-M	16.2	1.43
		21-F	15.8	1.86
		22-F	17.2	2.07
		23-F	11.7	2.09
		24-F	15.4	1.81
T-III	3.0	25-M	27.2	2.50
		26-M	18.7	1.97
		27-M	24.3	1.98
		28-M	20.3	2.26
		29-F	16.0	2.50
		30-F	15.6	2.23
		31-F	22.2	2.61
		32-F	20.0	2.10

TABLE XXIV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Gonads

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	5.4	0.495
		2-M	17.0	1.46
		3-M	15.5	1.30
		4-M	9.5	0.960
		5-F	0.586	0.0724
		6-F	0.621	0.0675
		7-F	0.509	0.0485
		8-F	0.489	0.0612
T-I	0.3	9-M	20.3	1.64
		10-M	18.9	1.62
	-	11-M	5.8	0.527
		12-M	22.5	2.04
		13-F	0.631	0.0770
		14-F	0.583	0.0678
		15-F	0.492	0.0656
		16-F	0.666	0.0659
T-II	1.0	17-M	11.2	1.10
		18-M	5.4	0.495
		19-M	16.5	1.60
		20-M	19.4	1.72
		21-F	0.492	0.0579
		22-F	0.581	0.0692
		23-F	0.492	0.0863
		24-F	0.614	0.0714
T-III	3.0	25-M	7.1	0.651
		26-M	11.0	1.16
		27-M	16.4	1.33
		28-M	7.0	0.778
		29-F	0.429	0.0660
		30-F	0.518	0.0710
		31-F	0.498	0.0566
		32-F	0.542	0.0564

TABLE XXV

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Adrenal Glands

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.942	0.0856
		2-M	0.956	0.0803
		3-M	1.088	0.0884
		4-M	0.966	0.0947
		5-F	0.745	0.0919
		6-F	0.831	0.0903
		7-F	0.941	0.0896
		8-F	0.867	0.108
T-I	0.3	9-M	1.026	0.0821
		10-M	0.978	0.0829
		11-M	0.910	0.0827
		12-M	1.011	0.0919
		13-F	0.744	0.0907
		14-F	1.092	0.127
		15-F	0.699	0.0932
		16-F	0.871	0.0862
T-II	1.0	17-M	0.881	0.0847
		18-M	1.049	0.0928
		19-M	0.962	0.0908
		20-M	1.030	0.0896
		21-F	0.910	0.107
		22-F	0.840	0.0999
		23-F	0.553	0.0971
		24-F	0.782	0.0909
T-III	3.0	25-M	0.977	0.0872
		26-M	0.918	0.0947
		27-M	1.173	0.0931
		28-M	0.824	0.0896
		29-F	0.594	0.0914
		30-F	0.709	0.0971
		31-F	0.889	0.101
		32-F	0.909	0.0947

TABLE XXVI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Thyroid Gland

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.981	0.0892
		2-M	1.102	0.0951
		3-M	1.201	0.0976
		4-M	1.166	0.114
		5-F	0.844	0.104
		6-F	0.907	0.0986
		7-F	1.005	0.0957
		8-F	0.804	0.100
T-I	0.3	9-M	1.178	0.0942
		10-M	1.034	0.0876
		11-M	1.038	0.0944
		12-M	1.075	0.0977
		13-F	0.736	0.0898
		14-F	0.801	0.0931
		15-F	0.818	0.109
		16-F	0.937	0.0928
T-II	1.0	17-M	1.023	0.0984
		18-M	1.053	0.0932
		19-M	0.939	0.0886
		20-M	1.288	0.112
		21-F	0.798	0.0939
		22-F	0.882	0.105
		23-F	0.511	0.0897
		24-F	0.837	0.0973
T-III	3.0	25-M	1.055	0.0942
		26-M	1.028	0.106
		27-M	1.236	0.0981
		28-M	0.869	0.0945
		29-F	0.612	0.0941
		30-F	0.737	0.101
		31-F	0.846	0.0962
		32-F	0.882	0.0919

TABLE XXVII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Pituitary Gland

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.083	0.00754
		2-M	0.098	0.00828
		3-M	0.085	0.00693
		4-M	0.072	0.00708
		5-F	0.064	0.00791
		6-F	0.077	0.00842
		7-F	0.073	0.00697
		8-F	0.052	0.00648
T-I	0.3	9-M	0.099	0.00790
		10-M	0.093	0.00786
		11-M	0.087	0.00793
		12-M	0.076	0.00690
		13-F	0.068	0.00831
		14-F	0.066	0.00767
		15-F	0.056	0.00748
		16-F	0.070	0.00693
T-II	1.0	17-M	0.075	0.00721
		18-M	0.079	0.00690
		19-M	0.086	0.00808
		20-M	0.091	0.00791
		21-F	0.058	0.00687
		22-F	0.063	0.00752
		23-F	0.042	0.00729
		24-F	0.074	0.00867
T-III	3.0	25-M	0.084	0.00747
		26-M	0.084	0.00862
		27-M	0.091	0.00721
		28-M	0.063	0.00688
		29-F	0.047	0.00720
		30-F	0.061	0.00842
		31-F	0.069	0.00785
		32-F	0.067	0.00698

2. Gross and Histologic Findings

The gross and histologic findings are presented in Tables XXVIII through XXXI. All tissues and organs not mentioned were normal. The grading system used is as follows:

\+ = minimal or slight
++ = mild
+++ = moderate
++++ = severe

IBT No. J749
Stauffer

I have completed a histopathologic evaluation of tissue from 32 dogs of IBT No. J749. There are no changes that can be attributed to the test material or the test procedure. All of the findings noted are attributed to spontaneous disease.

James F. Vondruska, D.V.M.
Senior Group Leader
Primate Toxicity

Reviewed & approved by:

Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

TABLE XXVIII

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
1-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Focal interstitial pneumonia	++
	Prostate	-	-	Chronic focal prostatitis	++
	Spleen	-	-	Hemosiderosis	+
2-M	Lungs	-	-	Chronic interstitial pneumonia	++
3-M	Heart	-	-	Congestion	+
	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Focal interstitial pneumonia	++
4-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
	Spleen	-	-	Hemosiderosis	+

TABLE XXVIII continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
5-F	Liver	-	-	Congestion	++
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
				Hyperemia	+
6-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Uterus	-	-	In estrus	-
7-F	Ovaries	-	-	Proestrus	-
	Liver	-	-	Congestion	++
8-F	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXIX

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
9-M	Brain	-	-	Calcified cyst	+
	Liver	-	-	Focal lymphoid infiltration	+
				Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Prostate	-	-	Chronic prostatitis	+++
10-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
11-M	Kidneys	-	-	Focal lymphoid infiltration	+
	Liver	-	-	Focal lymphoid infiltration	+
				Congestion	+
	Prostate	-	-	Chronic prostatitis	+++
12-M	Liver	-	-	Focal lymphoid infiltration	++
	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXIX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
13-F	Liver	-	-	Focal lymphoid infiltration	+
				Congestion	+
	Lungs	-	-	Focal interstitial pneumonia	+
14-F	Liver	-	-	Focal lymphoid infiltration	+
				Congestion	+
	Uterus	Enlarged	++	Diestrus	-
15-F	Liver	-	-	Congestion	++
	Lungs	-	-	Focal interstitial pneumonia	++
16-F	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXX

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group II: 1.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
17-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
18-M	Lungs	-	-	Chronic interstitial pneumonia	+
				Focal granulomatous infiltration	+
	Prostate	-	-	Chronic prostatitis	+++
19-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
20-M	Kidneys	-	-	Congestion	+
	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
21-F	Liver	-	-	Congestion	+
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	++
	Uterus	Enlarged	++	Diestrus	-

TABLE XXX continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group II: 1.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
22-F	Liver	-	-	Congestion	+
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
23-F	Liver	-	-	Congestion	+
				Focal lymphoid infiltration	+
24-F	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXXI

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
25-M	-	-	-	-	-
26-M	Liver	-	-	Focal lymphoid infiltration	++
	Lungs	-	-	Chronic interstitial pneumonia	+
27-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	++
28-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
29-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
				Focal granulomatous infiltration	+
30-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++

TABLE XXXI continued

TEST MATERIAL: Levair

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
31-F	Kidneys	-	-	Tubular casts	+
	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	++
32-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH KASAL IN BEAGLE DOGS

JUNE 30, 1972

IBT NO. J749

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

June 30, 1972

Mr. A. B. Lindquist, Manager
Product Registrations
Stauffer Chemical Company
1200 South 47th Street
Richmond, California 94804

Dear Mr. Lindquist:

Re: IBT No. J749 - 90/Day Subacute Oral Toxicity Study with Kasal in Beagle Dogs

We are submitting herewith our laboratory report dated June 30, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/mp

REPORT TO

STAUFFER CHEMICAL COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH
KASAL
IN BEAGLE DOGS

JUNE 30, 1972

IBT NO. J749

I. Introduction

A sample identified as Kasal was received from Stauffer Chemical Company for the purpose of conducting a 90-day subacute oral toxicity study using purebred beagle dogs. The following report presents the results of the investigation.

II. Summary

Ninety-day oral administration of Kasal to purebred beagel dogs at dietary levels of 0.3, 1.0 and 3.0 percent revealed no significant abnormalities in the following parameters:

Body Weights
Food Consumption
Behavioral Reactions
Mortality
Hematologic Studies
Blood Chemistry Studies
Urine Analyses
Organ Weights
Gross Pathologic Studies

However, histopathologic examination of kidney tissues revealed unusually large renal concretions in three of eight animals receiving 3.0 percent of Kasal. These concretions are thought to be related to the ingestion of test material. All other histopathologic changes can be attributed to naturally occurring disease.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: Kenneth Mastalski

Kenneth Mastalski, B.S.
Group Leader
Wedge's Creek Research Farm

Report approved by: Donald H Jenkins

Donald H. Jenkins, D.V.M.
Manager & Technical Director
Wedge's Creek Research Farm

M L Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

sjn:scm:slg:psh

Stauffer Chemical Company
Industrial Chemical Division

CERTIFICATE OF ANALYSIS

Material: Sodium Aluminum Phosphate, Basic Date 10/22/71
Common Name: KASAL

Identification: Material representing 20 bags Kasal coded 140-14230 Lot K-168

Analysis:

Determination		Food Chemical Codex Specifications
Assay (Al_2O_3)	10.9%	9.5% - 12.5%
Loss on Ignition	8.02%	9% maximum
Limits of Impurities		
Arsenic (As)	0.1 ppm	3 ppm maximum
Fluoride (F)	2.0 ppm	25 ppm maximum
Heavy Metals (as Pb)	<10 ppm	40 ppm maximum
Lead (Pb)	0.3 ppm	10 ppm maximum
P_2O_5	45.6%	-
pH (25% slurry)	9.2	-
Sieving:		
on 200 mesh	None	-
on 325 mesh	2.0%	-

This material complies with the specification of the Food Chemicals Codex.

R.S. Bryant

STATE OF NEW YORK
COUNTY OF NEW YORK

Sworn and subscribed to before me this
22nd day of October, 1971.

Patricia A. Pecquex

PATRICIA A. PECQUEX
NOTARY PUBLIC, State of New York
No. 24-0312690
Qualified in Kings County
Cert. filed in New York County
Commission Expires March 30, 19[illegible]

III. Procedure

A. Organization

The 90-day toxicity study utilized an untreated control group and three test groups, each consisting of eight purebred beagle dogs (four males and four females). The beagles were all eligible for A. K. C. registration and had been previously immunized against rabies, distemper, infectious canine hepatitis and leptospirosis.

All dogs were acquired from our own (IBTL) colony and were under observation for two weeks prior to the start of the investigation, during which time they were reimmunized and rendered clinically free of any existing parasitic infestation.

During the investigation, the selected animals were housed in kennels equipped with outside runs, four dogs of the same sex and group being accommodated in a single kennel.

The material to be tested, Kasal, was incorporated into a stock diet and fed to the dogs seven days a week at three graded dietary levels. The levels were 0.3, 1.0 and 3.0 percent of test material in the diet.

An outline of the test organization and diet composition is presented in Table I. A certificate of test material analysis is also presented.

TABLE I

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Test Organization and Diet Composition

Group	Number of Animals		Constituents of Diet	
	Male	Female	Dose Level (%)	Stock Ration* (g)
UC	4	4	None	100.0
T-I	4	4	0.3	99.7
T-II	4	4	1.0	99.0
T-III	4	4	3.0	97.0

* Golden Choice Meals, Adolph Coors Company, Denver, Colorado.

B. Parameters Investigated

Initially, the body weight of each dog in every group was determined and recorded. Thereafter, weighings were conducted weekly for the duration of the test.

At the beginning of each week, the appropriate dietary constituents for each of the groups were thoroughly blended in a Hobart mixer. Preweighed amounts were distributed into self-feeding units and maintained in excess of the animals' consumption. One such unit was available to the dogs in each kennel on an ad libitum basis 24 hours a day. At the end of each seven-day period, all unconsumed food was collected and weighed. Food consumption was then calculated and recorded. Water was available to the animals at all times.

The dogs were under observation during the investigation and were examined daily for clinical signs or symptoms indicative of systemic toxicity.

The following determinations were conducted upon each dog from the untreated control group and three test groups just prior to the inception of the study and after 42 and 84 days of testing.

Hematologic Studies

total leukocyte count
erythrocyte count
hemoglobin
hematocrit
differential leukocyte count

Blood Chemistry Studies

blood urea nitrogen
serum glucose
serum alkaline phosphatase
serum glutamic-oxalacetic transaminase
serum glutamic-pyruvic transaminase

Urine Analyses

albumin
glucose
pH
microscopic elements - leukocytes
erythrocytes
crystals

At the conclusion of the investigation, the dogs from each group were sacrificed by electric shock. All major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland and pituitary gland. The following tissues and organs excised from these animals were examined histologically (Hematoxylin-Eosin Stain):

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lungs
Lymph Nodes (cervical, mesenteric)
Muscle (skeletal)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (submaxillary)
Small Intestine (duodenum, jejunum, ileum)
Spinal Cord
Spleen
Stomach (cardia, fundus, pylorus)
Trachea
Thyroid Gland
Uterus
Urinary Bladder

IV. Results

A. Body Weight Data

The body weight data are presented in Tables II and III.

No significant deviations from normally expected body weight gains for dogs of this age were noted.

TABLE

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Males, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	1	5.5	10.0	10.1	10.1	9.8	10.3	10.0	11.0	10.8	10.6	10.8	11.1	11.1	10.9	11.0	1.0
		2	6.0	9.5	9.7	9.7	10.3	10.5	10.4	11.1	11.3	11.4	11.7	12.1	12.1	12.2	11.9	2.4
		3	6.0	10.2	10.1	10.5	10.8	11.2	11.6	11.5	11.8	11.7	12.2	12.5	12.3	11.9	12.3	2.1
		4	6.0	8.4	8.5	8.6	8.6	8.9	8.5	9.3	9.5	9.5	9.8	10.2	10.0	9.9	10.2	1.8
		Mean	5.9	9.5	9.6	9.7	9.9	10.2	10.1	10.7	10.8	10.8	11.1	11.5	11.4	11.2	11.4	1.9
T-I	0.3	9	5.5	8.0	8.5	8.7	8.5	8.7	8.8	8.7	9.1	9.1	9.2	9.2	9.7	9.5	9.3	1.3
		10	6.0	7.8	8.1	8.0	8.3	8.5	8.7	8.9	8.9	8.8	9.1	8.9	9.1	8.7	9.0	1.2
		11	6.0	8.7	9.2	9.4	9.5	9.5	9.8	9.8	10.5	10.2	10.8	10.7	10.8	10.7	10.9	2.2
		12	6.0	8.2	8.5	9.0	9.2	9.6	9.8	10.0	10.5	10.4	10.9	10.8	11.2	11.0	11.0	2.8
		Mean	5.9	8.2	8.6	8.8	8.9	9.1	9.3	9.4	9.8	9.6	10.0	9.9	10.2	10.0	10.0	1.8
T-II	1.0	17	5.5	10.0	10.3	10.8	11.1	11.3	11.6	11.8	12.1	12.1	12.0	12.4	12.7	12.4	12.9	2.9
		18	6.0	10.3	10.6	11.0	11.2	11.3	11.5	11.5	11.7	11.3	11.3	11.3	10.9	11.0	11.1	0.8
		19	6.0	8.5	8.6	9.0	9.4	9.4	10.0	9.9	10.4	10.3	10.2	10.5	10.7	10.6	10.5	2.0
		20	6.0	8.2	8.9	9.1	9.5	9.6	10.1	10.4	10.5	10.8	11.1	10.9	11.0	10.7	10.8	2.6
		Mean	5.9	9.2	9.6	10.0	10.3	10.4	10.8	10.9	11.2	11.1	11.2	11.3	11.3	11.2	11.3	2.1
T-III	3.0	25	5.8	6.0	6.3	6.6	6.8	7.0	7.2	7.3	7.7	7.7	7.8	8.4	8.6	8.2	8.3	2.3
		26	6.0	8.5	9.0	9.5	9.6	9.5	9.8	10.2	10.1	10.6	10.8	11.1	11.4	10.9	11.4	2.9
		27	6.0	8.5	8.4	8.8	9.0	9.4	9.3	9.5	9.8	9.8	9.9	9.9	10.0	9.8	10.0	1.5
		28	6.0	7.2	7.5	7.7	7.7	7.8	8.3	8.3	8.6	8.6	8.7	8.9	8.9	8.7	8.8	1.6
		Mean	6.0	7.6	7.8	8.2	8.3	8.4	8.6	8.8	9.0	9.2	9.3	9.6	9.7	9.4	9.6	2.0

TABLE III

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Females, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	5	6.0	6.6	6.9	7.1	7.3	7.3	7.4	7.8	7.8	7.8	8.0	8.1	8.2	8.0	8.1	1.5
		6	6.0	7.9	8.1	8.4	8.3	8.4	8.1	9.1	8.9	8.9	9.1	9.1	9.3	9.2	9.2	1.3
		7	6.0	8.0	8.4	8.8	8.7	9.0	9.0	9.7	10.0	9.7	10.2	10.1	10.6	10.5	10.5	2.5
		8	6.0	6.7	6.6	6.9	7.1	7.4	7.1	7.7	7.8	7.8	8.0	8.2	8.2	8.0	8.0	1.3
		Mean	6.0	7.3	7.5	7.8	7.8	8.0	7.9	8.6	8.6	8.6	8.8	8.9	9.1	8.9	9.0	1.7
T-I	0.3	13	5.5	6.0	6.2	6.5	6.8	6.7	6.8	6.8	6.8	6.9	6.9	7.1	7.1	6.9	6.8	0.8
		14	5.5	7.8	7.1	7.5	7.8	7.9	7.9	8.2	8.0	8.3	8.4	8.5	8.6	8.4	8.5	0.7
		15	5.5	6.0	6.4	6.9	7.3	7.2	7.5	7.7	8.3	8.4	8.6	8.8	9.1	8.8	8.8	2.8
		16	6.0	10.5	10.7	11.4	11.6	11.5	11.8	11.8	12.0	12.0	12.3	12.6	12.6	12.4	12.1	1.6
		Mean	5.6	7.6	7.6	8.1	8.4	8.3	8.5	8.6	8.8	8.9	9.0	9.2	9.4	9.1	9.0	1.4
T-II	1.0	21	5.5	5.2	5.1	5.6	5.6	5.6	5.7	5.7	5.8	5.9	6.0	6.2	6.1	5.9	5.9	0.7
		22	5.5	5.9	5.9	6.4	6.4	6.4	6.6	6.6	6.8	6.9	7.1	7.1	7.2	6.9	7.1	1.2
		23	5.5	4.8	5.0	5.3	5.4	5.4	5.7	5.5	5.6	5.7	5.7	5.6	6.0	5.8	5.6	0.8
		24	6.0	6.1	6.0	6.7	7.1	7.3	7.7	7.7	8.2	8.3	8.9	9.0	9.1	8.8	9.1	3.0
		Mean	5.6	5.5	5.5	6.0	6.1	6.2	6.4	6.4	6.6	6.7	6.9	7.0	7.1	6.8	6.9	1.4
T-III	3.0	29	5.5	7.5	8.0	8.6	8.8	9.1	9.7	9.9	10.0	10.1	10.4	10.6	10.6	10.4	10.6	3.1
		30	5.5	4.8	4.9	5.0	5.1	5.0	5.3	5.3	5.4	5.4	5.5	5.5	5.5	5.4	5.3	0.5
		31	5.5	9.0	8.9	9.4	9.4	9.9	10.0	10.1	10.3	10.2	10.5	10.6	10.6	10.4	10.5	1.5
		32	5.5	7.0	7.0	7.3	7.4	7.9	7.6	7.7	7.6	7.5	7.8	8.0	8.0	7.7	7.7	0.7
		Mean	5.5	7.1	7.2	7.6	7.7	8.0	8.2	8.2	8.3	8.3	8.6	8.7	8.7	8.5	8.5	1.4

B. Food Consumption Data

Food consumption data are presented in Table IV.

There is no significant difference between the untreated control group and the three test groups.

TABLE IV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Food Consumption Data

		Mean Food Consumed During Week Indicated (grams/day)							
	Sex:	Males				Females			
	Group:	UC	T-I	T-II	T-III	UC	T-I	T-II	T-III
Week	Dietary Level (%):	None	0.3	1.0	3.0	None	0.3	1.0	3.0
1		352	375	388	401	380	451	436	385
2		361	381	385	430	417	407	458	418
3		366	362	359	392	397	378	410	386
4		338	371	353	342	366	382	407	375
5		356	358	336	363	399	359	391	369
6		348	335	332	366	375	352	394	351
7		319	347	328	364	343	369	398	352
8		285	305	277	323	323	331	358	346
9		315	335	268	325	321	361	377	363
10		333	303	274	328	362	370	361	356
11		300	336	321	317	341	332	334	330
12		286	268	261	287	298	302	279	288
13		281	338	304	346	285	344	423	350
Mean		326	340	322	353	354	364	387	359

C. Reactions

No untoward behavioral reactions were recorded during the investigation.

D. Mortality

No fatalities occurred during the investigation.

E. Hematologic Studies

The results of these determinations are presented in Tables V through IX.

No significant abnormalities were noted at any levels tested.

TABLE V

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Total Leukocyte Count, thousands/mm^3

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	14.2	12.3	9.3
		2-M	19.9	17.2	14.7
		3-M	17.0	18.0	15.4
		4-M	16.4	15.2	10.7
		5-F	12.9	14.6	11.1
		6-F	12.2	14.0	11.9
		7-F	14.0	15.2	11.9
		8-F	9.9	12.7	12.7
T-I	0.3	9-M	11.5	13.7	9.5
		10-M	13.7	13.9	10.1
		11-M	15.5	16.5	15.5
		12-M	26.7	19.2	18.4
		13-F	12.7	14.3	10.6
		14-F	18.9	20.2	12.4
		15-F	17.6	18.0	12.6
		16-F	16.7	17.8	12.4
T-II	1.0	17-M	15.7	18.7	14.5
		18-M	15.1	13.8	10.7
		19-M	17.3	15.5	13.9
		20-M	14.7	15.2	13.3
		21-F	10.3	12.0	10.4
		22-F	16.2	13.2	10.9
		23-F	15.1	15.0	12.3
		24-F	13.7	11.9	10.6
T-III	3.0	25-M	17.7	17.5	11.4
		26-M	17.4	16.8	16.0
		27-M	16.8	13.7	10.5
		28-M	14.1	12.8	12.9
		29-F	9.3	10.5	8.8
		30-F	11.4	14.4	11.0
		31-F	11.9	12.2	11.5
		32-F	16.3	16.7	13.4

TABLE VI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Erythrocyte Count, millions/mm^3

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	6.57	6.64	7.56
		2-M	7.06	6.60	6.89
		3-M	6.45	6.69	6.79
		4-M	5.68	6.06	6.37
		5-F	6.58	6.57	7.09
		6-F	6.50	6.98	6.92
		7-F	6.48	7.10	6.98
		8-F	5.89	6.48	6.76
T-I	0.3	9-M	6.34	6.55	6.89
		10-M	6.99	6.91	7.39
		11-M	6.26	6.31	7.17
		12-M	6.28	6.18	7.25
		13-F	6.33	6.34	6.87
		14-F	6.57	6.76	7.36
		15-F	5.47	5.97	6.44
		16-F	6.48	6.81	7.40
T-II	1.0	17-M	6.44	6.53	7.14
		18-M	6.01	5.52	6.27
		19-M	6.12	6.54	7.17
		20-M	5.92	5.82	6.93
		21-F	6.22	6.52	6.98
		22-F	6.09	6.64	7.14
		23-F	6.57	7.01	7.40
		24-F	5.43	5.75	6.84
T-III	3.0	25-M	5.62	5.83	6.73
		26-M	5.61	5.73	6.38
		27-M	5.88	6.21	7.04
		28-M	6.18	6.11	6.49
		29-F	6.72	6.76	7.61
		30-F	6.09	6.23	6.90
		31-F	6.41	6.39	6.85
		32-F	6.92	6.82	7.44

TABLE VII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Hemoglobin, gm/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15.4	15.5	16.8
		2-M	16.3	15.7	16.0
		3-M	15.5	16.6	16.3
		4-M	14.2	14.8	15.7
		5-F	14.5	15.4	16.6
		6-F	15.3	16.8	16.1
		7-F	15.2	17.3	16.6
		8-F	14.1	15.7	16.1
T-I	0.3	9-M	15.3	15.7	16.6
		10-M	16.0	15.6	17.5
		11-M	15.0	15.8	17.9
		12-M	14.2	14.4	16.6
		13-F	15.1	15.4	16.2
		14-F	15.6	16.0	17.3
		15-F	12.9	14.3	15.4
		16-F	15.4	16.7	17.9
T-II	1.0	17-M	15.3	15.5	17.3
		18-M	13.9	12.6	14.5
		19-M	14.2	15.0	16.6
		20-M	13.7	13.7	15.8
		21-F	14.8	15.6	16.8
		22-F	14.9	16.0	17.2
		23-F	15.0	16.7	17.8
		24-F	12.7	13.9	16.1
T-III	3.0	25-M	12.9	14.1	16.1
		26-M	13.0	13.5	14.8
		27-M	13.9	14.9	16.7
		28-M	14.4	14.3	15.1
		29-F	14.8	15.6	17.8
		30-F	14.3	15.0	16.9
		31-F	14.5	14.8	15.3
		32-F	16.1	16.1	18.2

TABLE VIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Hematocrit, percent

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	43.7	46.4	46.3
		2-M	47.0	45.7	43.2
		3-M	44.9	48.5	44.4
		4-M	39.0	43.7	41.8
		5-F	41.0	44.5	44.4
		6-F	43.0	48.9	44.4
		7-F	43.3	50.4	44.3
		8-F	40.2	46.2	44.8
T-I	0.3	9-M	44.7	48.5	46.1
		10-M	46.3	48.3	47.2
		11-M	42.7	46.4	47.6
		12-M	41.4	43.1	45.9
		13-F	41.7	44.7	44.0
		14-F	44.3	48.7	48.0
		15-F	36.8	42.7	41.6
		16-F	44.6	50.9	49.6
T-II	1.0	17-M	43.7	47.6	47.2
		18-M	39.6	38.4	39.4
		19-M	40.2	44.8	44.7
		20-M	38.1	40.8	43.2
		21-F	41.4	45.5	44.9
		22-F	41.8	47.4	46.4
		23-F	43.6	50.0	48.1
		24-F	35.7	41.0	44.5
T-III	3.0	25-M	37.6	41.2	43.1
		26-M	36.4	39.7	40.4
		27-M	38.5	43.3	43.8
		28-M	40.4	42.5	40.6
		29-F	42.2	45.7	46.1
		30-F	40.1	44.1	44.7
		31-F	40.9	43.0	42.0
		32-F	46.0	49.2	48.5

TABLE IX

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Neutrophils

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	66	74	56
		2-M	77	70	69
		3-M	49	66	57
		4-M	56	70	68
		5-F	65	59	66
		6-F	73	59	73
		7-F	46	61	63
		8-F	31	58	68
T-I	0.3	9-M	53	63	54
		10-M	53	54	52
		11-M	42	60	66
		12-M	66	63	62
		13-F	53	68	64
		14-F	50	59	51
		15-F	55	61	54
		16-F	60	64	63
T-II	1.0	17-M	53	54	50
		18-M	66	60	66
		19-M	54	46	60
		20-M	45	59	64
		21-F	43	60	58
		22-F	62	49	63
		23-F	54	50	66
		24-F	34	50	49
T-III	3.0	25-M	56	63	62
		26-M	33	69	58
		27-M	50	60	55
		28-M	40	56	60
		29-F	58	59	52
		30-F	47	58	59
		31-F	28	62	49
		32-F	60	64	63

TABLE IX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Lymphocytes

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	28	19	36
		2-M	17	21	28
		3-M	39	25	33
		4-M	39	21	26
		5-F	26	31	29
		6-F	17	29	25
		7-F	45	27	30
		8-F	64	38	21
T-I	0.3	9-M	42	26	36
		10-M	40	35	36
		11-M	49	32	30
		12-M	26	29	30
		13-F	41	23	31
		14-F	49	38	46
		15-F	43	35	43
		16-F	32	33	33
T-II	1.0	17-M	43	40	45
		18-M	32	36	32
		19-M	41	46	38
		20-M	54	39	31
		21-F	55	37	38
		22-F	35	31	33
		23-F	39	39	30
		24-F	62	42	49
T-III	3.0	25-M	39	26	34
		26-M	60	24	37
		27-M	38	30	37
		28-M	54	43	37
		29-F	39	37	42
		30-F	48	34	34
		31-F	66	33	48
		32-F	39	28	31

TABLE IX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Monocytes

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	2	4	3
		2-M	3	1	1
		3-M	2	2	0
		4-M	0	4	1
		5-F	0	3	1
		6-F	2	5	0
		7-F	3	5	0
		8-F	0	0	2
T-I	0.3	9-M	0	2	3
		10-M	1	3	1
		11-M	2	1	1
		12-M	3	0	1
		13-F	2	4	1
		14-F	1	2	0
		15-F	1	2	2
		16-F	7	3	3
T-II	1.0	17-M	1	2	1
		18-M	0	2	2
		19-M	3	5	1
		20-M	0	2	1
		21-F	2	2	3
		22-F	2	5	3
		23-F	1	3	1
		24-F	1	2	1
T-III	3.0	25-M	2	1	2
		26-M	2	2	0
		27-M	2	4	2
		28-M	2	1	1
		29-F	2	1	2
		30-F	1	3	2
		31-F	1	2	2
		32-F	1	2	2

TABLE IX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Eosinophils

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	4	3	5
		2-M	3	8	2
		3-M	10	7	10
		4-M	5	5	5
		5-F	9	7	4
		6-F	8	7	2
		7-F	6	7	7
		8-F	5	4	9
T-I	0.3	9-M	5	9	7
		10-M	6	8	11
		11-M	7	7	3
		12-M	5	8	7
		13-F	4	5	4
		14-F	0	1	3
		15-F	1	2	1
		16-F	1	0	1
T-II	1.0	17-M	3	4	4
		18-M	2	3	0
		19-M	2	2	1
		20-M	1	0	4
		21-F	0	1	1
		22-F	1	15	1
		23-F	6	8	3
		24-F	3	6	1
T-III	3.0	25-M	3	10	2
		26-M	5	5	5
		27-M	10	6	6
		28-M	4	0	2
		29-F	1	3	4
		30-F	4	5	5
		31-F	5	3	1
		32-F	0	6	4

TABLE IX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Differential Leukocyte Count, percent - Basophils

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	0
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

F. Blood Chemistry Studies

The results of these determinations are presented in Tables X through XIV.

There is no significant difference between the untreated control group and the three test groups.

TABLE X

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Blood Urea Nitrogen, mg/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	20	20	14
		2-M	13	15	12
		3-M	16	20	14
		4-M	16	23	12
		5-F	15	19	11
		6-F	10	15	11
		7-F	11	12	10
		8-F	16	19	12
T-I	0.3	9-M	21	19	11
		10-M	13	13	16
		11-M	13	12	16
		12-M	22	19	17
		13-F	17	17	13
		14-F	17	19	14
		15-F	17	20	12
		16-F	10	15	12
T-II	1.0	17-M	17	16	14
		18-M	21	19	12
		19-M	15	19	15
		20-M	19	15	13
		21-F	19	16	14
		22-F	17	20	12
		23-F	18	13	14
		24-F	13	18	10
T-III	3.0	25-M	16	22	19
		26-M	21	12	13
		27-M	15	13	13
		28-M	14	15	11
		29-F	15	13	9
		30-F	15	14	13
		31-F	14	17	13
		32-F	21	14	14

TABLE XI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glucose, mg/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	90	112	105
		2-M	127	125	108
		3-M	118	125	113
		4-M	115	103	97
		5-F	104	113	104
		6-F	121	123	99
		7-F	97	103	102
		8-F	93	108	96
T-I	0.3	9-M	121	115	111
		10-M	124	117	108
		11-M	124	110	99
		12-M	120	112	110
		13-F	106	101	91
		14-F	104	103	92
		15-F	88	103	96
		16-F	109	106	101
T-II	1.0	17-M	123	110	101
		18-M	102	100	94
		19-M	109	101	94
		20-M	112	105	99
		21-F	102	97	91
		22-F	112	97	96
		23-F	95	93	94
		24-F	109	106	97
T-III	3.0	25-M	93	77	85
		26-M	127	113	119
		27-M	112	106	97
		28-M	121	98	99
		29-F	112	115	110
		30-F	102	109	94
		31-F	104	106	94
		32-F	121	112	107

TABLE XII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Alkaline Phosphatase, King-Armstrong Units/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7.06	6.63	5.23
		2-M	6.87	6.34	5.23
		3-M	6.68	6.78	5.06
		4-M	17.33	25.62	13.82
		5-F	9.45	9.10	7.36
		6-F	7.83	8.14	6.81
		7-F	7.44	7.52	6.10
		8-F	4.51	5.23	3.74
T-I	0.3	9-M	6.30	8.94	6.28
		10-M	9.24	11.18	9.28
		11-M	6.68	6.34	5.40
		12-M	4.51	4.30	3.90
		13-F	7.06	7.27	6.45
		14-F	6.30	6.78	5.40
		15-F	5.94	7.52	6.28
		16-F	8.23	14.79	11.56
T-II	1.0	17-M	5.57	5.64	3.74
		18-M	8.63	11.18	10.09
		19-M	10.29	11.18	7.92
		20-M	10.29	10.47	9.08
		21-F	6.87	6.34	4.89
		22-F	7.06	8.61	5.75
		23-F	6.30	6.20	4.89
		24-F	9.04	9.43	7.92
T-III	3.0	25-M	9.87	7.83	6.99
		26-M	7.44	7.52	6.63
		27-M	9.45	9.77	7.73
		28-M	7.44	8.14	8.69
		29-F	6.30	6.63	4.06
		30-F	11.16	13.10	10.29
		31-F	7.83	8.30	6.99
		32-F	6.68	6.06	4.23

TABLE XIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Oxalacetic Transaminase, Dade Units/ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	17	16	18
		2-M	24	21	21
		3-M	24	17	19
		4-M	39	20	27
		5-F	32	20	20
		6-F	14	20	18
		7-F	27	22	15
		8-F	14	15	14
T-I	0.3	9-M	19	26	14
		10-M	37	23	13
		11-M	52	22	16
		12-M	24	19	14
		13-F	12	22	13
		14-F	8	15	12
		15-F	16	13	11
		16-F	18	23	17
T-II	1.0	17-M	22	23	13
		18-M	29	25	16
		19-M	39	32	23
		20-M	29	31	20
		21-F	22	19	14
		22-F	17	19	14
		23-F	24	22	24
		24-F	17	27	23
T-III	3.0	25-M	32	23	24
		26-M	22	24	16
		27-M	22	22	18
		28-M	30	25	15
		29-F	17	26	15
		30-F	30	31	21
		31-F	21	31	18
		32-F	22	25	18

TABLE XIV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Pyruvic Transaminase, Dade Units/ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15	15	17
		2-M	66	19	27
		3-M	15	16	24
		4-M	15	15	27
		5-F	15	14	15
		6-F	15	15	16
		7-F	15	15	16
		8-F	15	15	18
T-I	0.3	9-M	15	15	21
		10-M	24	16	21
		11-M	24	15	19
		12-M	15	19	30
		13-F	16	17	26
		14-F	15	15	15
		15-F	15	15	15
		16-F	15	15	18
T-II	1.0	17-M	15	15	24
		18-M	15	15	18
		19-M	16	16	31
		20-M	15	15	17
		21-F	15	14	15
		22-F	15	15	20
		23-F	15	17	17
		24-F	15	14	15
T-III	3.0	25-M	15	15	17
		26-M	15	19	39
		27-M	15	19	23
		28-M	16	15	24
		29-F	14	14	15
		30-F	15	16	23
		31-F	15	23	28
		32-F	15	17	24

G. Urine Analyses

The results of the urine analyses are presented in Tables XV through XVIII.

Urinalysis revealed no significant abnormalities at any of the levels tested.

TABLE XV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Albumin, mg/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	10	0	0
		2-M	10	0	0
		3-M	0	0	0
		4-M	10	10	0
		5-F	0	0	0
		6-F	10	11-50	0
		7-F	10	0	11-50
		8-F	0	0	0
T-I	0.3	9-M	10	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	10	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	10	0
		20-M	0	0	0
		21-F	10	0	0
		22-F	0	0	11-50
		23-F	0	0	10
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	11-50	11-50
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

TABLE XVI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Glucose, mg/100 ml

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	0
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

TABLE XVII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: pH

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7	6	7
		2-M	8	7	6
		3-M	7	7	5
		4-M	7	6	8
		5-F	6	8	8
		6-F	8	7	7
		7-F	8	7	6
		8-F	6	8	7
T-I	0.3	9-M	6	6	8
		10-M	6	6	8
		11-M	6	7	8
		12-M	6	5	8
		13-F	6	6	7
		14-F	6	6	6
		15-F	6	5	6
		16-F	6	7	6
T-II	1.0	17-M	6	6	5
		18-M	6	7	7
		19-M	6	6	7
		20-M	6	7	6
		21-F	6	6	5
		22-F	6	6	5
		23-F	6	8	7
		24-F	6	7	8
T-III	3.0	25-M	7	6	7
		26-M	7	6	7
		27-M	8	6	7
		28-M	7	6	6
		29-F	6	5	5
		30-F	6	7	7
		31-F	6	6	8
		32-F	6	6	5

TABLE XVIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements, average per high power field - Leukocytes

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	20	7	0
		8-F	3	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	4	0	2
		14-F	0	0	0
		15-F	6	3	0
		16-F	0	8	4
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	4	0	0
		22-F	0	0	5
		23-F	8	0	10
		24-F	0	0	10
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	3	0
		30-F	4	0	10
		31-F	4	0	0
		32-F	0	0	0

TABLE XVIII continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements,
average per high power field - Erythrocytes

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	25*
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	4	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

* in estrus

TABLE XVIII continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements,
average per high power field - Crystals

Group	Dietary Level (percent)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	1-3	1-3	1-3
		2-M	1-3	1-3	1-3
		3-M	1-3	1-3	1-3
		4-M	1-3	1-3	1-3
		5-F	1-3	1-3	1-3
		6-F	1-3	1-3	1-3
		7-F	1-3	1-3	1-3
		8-F	1-3	1-3	1-3
T-I	0.3	9-M	1-3	1-3	1-3
		10-M	1-3	1-3	1-3
		11-M	1-3	1-3	1-3
		12-M	1-3	1-3	1-3
		13-F	1-3	1-3	1-3
		14-F	1-3	1-3	1-3
		15-F	1-3	1-3	1-3
		16-F	1-3	1-3	1-3
T-II	1.0	17-M	1-3	1-3	1-3
		18-M	1-3	1-3	1-3
		19-M	1-3	1-3	1-3
		20-M	1-3	1-3	1-3
		21-F	4	1-3	1-3
		22-F	1-3	1-3	1-3
		23-F	1-3	1-3	1-3
		24-F	1-3	1-3	1-3
T-III	3.0	25-M	4	1-3	1-3
		26-M	1-3	1-3	1-3
		27-M	4	1-3	1-3
		28-M	4	1-3	1-3
		29-F	1-3	1-3	1-3
		30-F	1-3	1-3	4
		31-F	1-3	1-3	1-3
		32-F	1-3	1-3	1-3

H. Pathologic Findings

1. Organ Weight Data

The organ weight data are presented in Tables XIX through XXVII.

No significant abnormalities were noted among any levels tested.

TABLE XIX

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Liver

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	400.0	36.7
		2-M	402.1	34.7
		3-M	445.9	37.5
		4-M	364.4	36.8
		5-F	273.9	34.8
		6-F	301.7	33.9
		7-F	439.2	42.2
		8-F	294.4	37.7
T-I	0.3	9-M	324.9	35.7
		10-M	316.8	35.2
		11-M	373.9	34.9
		12-M	363.8	34.6
		13-F	222.3	33.7
		14-F	270.5	32.2
		15-F	355.0	39.0
		16-F	473.4	37.9
T-II	1.0	17-M	409.2	33.8
		18-M	413.2	37.6
		19-M	373.2	34.6
		20-M	377.6	34.3
		21-F	211.1	35.8
		22-F	249.6	35.2
		23-F	211.8	38.5
		24-F	316.8	35.2
T-III	3.0	25-M	290.6	36.3
		26-M	414.6	37.7
		27-M	372.0	38.8
		28-M	345.7	39.3
		29-F	403.7	38.4
		30-F	200.8	36.5
		31-F	354.4	34.1
		32-F	280.9	36.0

TABLE XX

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Kidneys

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	68.7	6.30
		2-M	69.7	6.01
		3-M	68.4	5.75
		4-M	71.4	7.21
		5-F	48.5	6.06
		6-F	57.2	6.43
		7-F	64.8	6.23
		8-F	48.4	6.20
T-I	0.3	9-M	66.2	7.28
		10-M	49.8	5.53
		11-M	69.8	6.52
		12-M	66.2	6.30
		13-F	41.6	6.30
		14-F	48.7	5.80
		15-F	56.7	6.23
		16-F	63.8	5.10
T-II	1.0	17-M	74.6	6.16
		18-M	69.8	6.34
		19-M	63.8	5.91
		20-M	72.0	6.54
		21-F	39.2	6.64
		22-F	40.2	5.66
		23-F	35.3	6.42
		24-F	54.7	6.08
T-III	3.0	25-M	55.9	6.99
		26-M	79.0	7.18
		27-M	62.5	6.51
		28-M	59.7	6.78
		29-F	59.5	5.67
		30-F	38.5	7.00
		31-F	55.5	5.34
		32-F	50.7	6.50

TABLE XXI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Heart

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	91.9	8.43
		2-M	99.3	8.56
		3-M	107.6	9.04
		4-M	86.1	8.70
		5-F	74.3	9.29
		6-F	92.7	10.4
		7-F	78.3	7.53
		8-F	70.9	9.09
T-I	0.3	9-M	81.1	8.91
		10-M	74.0	8.22
		11-M	84.5	7.90
		12-M	102.0	9.71
		13-F	59.8	9.06
		14-F	70.4	8.38
		15-F	57.8	6.35
		16-F	108.2	8.66
T-II	1.0	17-M	113.5	9.38
		18-M	104.5	9.50
		19-M	99.8	9.24
		20-M	89.2	8.11
		21-F	56.0	9.49
		22-F	63.1	8.89
		23-F	51.8	9.42
		24-F	69.5	7.72
T-III	3.0	25-M	70.0	8.75
		26-M	93.0	8.46
		27-M	88.4	9.21
		28-M	75.7	8.60
		29-F	97.7	9.30
		30-F	58.5	10.6
		31-F	81.5	7.84
		32-F	67.5	8.65

TABLE XXII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Brain

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	76.7	7.04
		2-M	86.5	7.46
		3-M	90.5	7.60
		4-M	78.6	7.94
		5-F	82.0	10.2
		6-F	85.5	9.48
		7-F	78.0	7.50
		8-F	78.3	10.0
T-I	0.3	9-M	80.0	8.79
		10-M	77.5	8.61
		11-M	72.1	6.74
		12-M	87.2	8.30
		13-F	75.9	11.5
		14-F	72.7	8.66
		15-F	70.9	7.79
		16-F	80.2	6.42
T-II	1.0	17-M	86.4	7.14
		18-M	81.4	7.40
		19-M	76.0	7.04
		20-M	85.6	7.78
		21-F	75.2	12.7
		22-F	66.4	9.35
		23-F	69.5	12.6
		24-F	64.4	7.16
T-III	3.0	25-M	66.6	8.32
		26-M	78.8	7.16
		27-M	84.0	8.75
		28-M	67.4	7.66
		29-F	77.5	7.38
		30-F	64.2	11.7
		31-F	78.3	7.53
		32-F	68.5	8.78

TABLE XXIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Spleen

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	22.2	2.04
		2-M	16.2	1.40
		3-M	35.8	3.01
		4-M	16.2	1.64
		5-F	16.9	2.11
		6-F	23.6	2.65
		7-F	25.4	2.44
		8-F	18.5	2.37
T-I	0.3	9-M	15.5	1.70
		10-M	19.2	2.13
		11-M	22.0	2.06
		12-M	27.6	2.63
		13-F	9.9	1.50
		14-F	19.6	2.33
		15-F	16.4	1.80
		16-F	41.8	3.34
T-II	1.0	17-M	25.0	2.06
		18-M	21.4	1.94
		19-M	31.3	2.90
		20-M	21.4	1.94
		21-F	10.0	1.70
		22-F	14.5	2.04
		23-F	16.0	2.91
		24-F	18.0	2.00
T-III	3.0	25-M	13.8	1.72
		26-M	16.7	1.52
		27-M	20.2	2.10
		28-M	23.1	2.62
		29-F	19.4	1.85
		30-F	14.8	2.69
		31-F	20.6	1.98
		32-F	16.4	2.10

TABLE XXIV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Gonads

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	5.4	0.495
		2-M	17.0	1.46
		3-M	15.5	1.30
		4-M	9.5	0.960
		5-F	0.586	0.0724
		6-F	0.621	0.0675
		7-F	0.509	0.0485
		8-F	0.489	0.0612
T-I	0.3	9-M	18.0	1.98
		10-M	14.5	1.61
		11-M	11.0	1.03
		12-M	23.1	2.20
		13-F	0.437	0.0643
		14-F	0.494	0.0581
		15-F	0.634	0.0721
		16-F	0.768	0.0635
T-II	1.0	17-M	10.8	0.893
		18-M	18.1	1.64
		19-M	14.1	1.31
		20-M	6.0	0.545
		21-F	0.477	0.0809
		22-F	0.462	0.0651
		23-F	0.387	0.0691
		24-F	0.530	0.0582
T-III	3.0	25-M	10.8	1.35
		26-M	15.5	1.41
		27-M	16.2	1.69
		28-M	14.1	1.60
		29-F	0.750	0.0708
		30-F	0.336	0.0635
		31-F	0.626	0.0596
		32-F	0.494	0.0641

TABLE XXV

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Adrenal Glands

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	0.942	0.0856
		2-M	0.956	0.0803
		3-M	1.088	0.0884
		4-M	0.966	0.0947
		5-F	0.745	0.0919
		6-F	0.831	0.0903
		7-F	0.941	0.0896
		8-F	0.867	0.108
T-I	0.3	9-M	0.811	0.0872
		10-M	0.816	0.0907
		11-M	0.950	0.0872
		12-M	0.978	0.0889
		13-F	0.619	0.0911
		14-F	0.867	0.102
		15-F	0.847	0.0963
		16-F	1.026	0.0848
T-II	1.0	17-M	1.20	0.0932
		18-M	0.972	0.0876
		19-M	0.866	0.0825
		20-M	0.917	0.0849
		21-F	0.568	0.0963
		22-F	0.597	0.0841
		23-F	0.556	0.0992
		24-F	0.831	0.0913
T-III	3.0	25-M	0.737	0.0888
		26-M	1.046	0.0917
		27-M	0.010	0.101
		28-M	0.788	0.896
		29-F	0.011	0.0954
		30-F	0.489	0.0923
		31-F	0.864	0.0823
		32-F	0.669	0.0869

TABLE XXVI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Thyroid Gland

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	0.981	0.0892
		2-M	1.102	0.0951
		3-M	1.201	0.0976
		4-M	1.166	0.114
		5-F	0.844	0.104
		6-F	0.907	0.0986
		7-F	1.005	0.0957
		8-F	0.804	0.100
T-I	0.3	9-M	0.866	0.0931
		10-M	0.767	0.0852
		11-M	0.994	0.0912
		12-M	1.042	0.0947
		13-F	0.741	0.109
		14-F	0.745	0.0876
		15-F	0.823	0.0935
		16-F	1.106	0.0914
T-II	1.0	17-M	1.214	0.0941
		18-M	0.997	0.0898
		19-M	0.969	0.0923
		20-M	1.064	0.0985
		21-F	0.529	0.0896
		22-F	0.802	0.113
		23-F	0.518	0.0925
		24-F	0.808	0.0888
T-III	3.0	25-M	0.872	0.105
		26-M	1.080	0.947
		27-M	0.918	0.0918
		28-M	0.778	0.0895
		29-F	0.976	0.0921
		30-F	0.501	0.0946
		31-F	0.938	0.0893
		32-F	0.839	0.109

TABLE XXVII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Pituitary Gland

Group	Dietary Level (percent)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1,000 g)
UC	None	1-M	0.083	0.00754
		2-M	0.098	0.00828
		3-M	0.085	0.00693
		4-M	0.072	0.00708
		5-F	0.064	0.00791
		6-F	0.077	0.00842
		7-F	0.073	0.00697
		8-F	0.052	0.00648
T-I	0.3	9-M	0.067	0.00721
		10-M	0.062	0.00693
		11-M	0.089	0.00713
		12-M	0.078	0.00713
		13-F	0.045	0.00656
		14-F	0.070	0.00821
		15-F	0.064	0.00727
		16-F	0.077	0.00639
T-II	1.0	17-M	0.096	0.00747
		18-M	0.090	0.00813
		19-M	0.073	0.00692
		20-M	0.084	0.00777
		21-F	0.044	0.00743
		22-F	0.062	0.00671
		23-F	0.046	0.00814
		24-F	0.072	0.00791
T-III	3.0	25-M	0.057	0.00682
		26-M	0.086	0.00757
		27-M	0.084	0.00841
		28-M	0.071	0.00797
		29-F	0.069	0.00648
		30-F	0.044	0.00839
		31-F	0.074	0.00709
		32-F	0.070	0.00913

2. Gross and Histologic Findings

The gross and histologic findings are presented in Tables XXVIII through XXXI. All tissues and organs not mentioned were normal.

The grading system used is as follows:

\+ = minimal or slight
++ = mild
+++ = moderate
++++ = severe
+++++ = extreme

IBT No. J749
Stauffer

I have completed a histopathologic evaluation of sections of tissues from dogs of the above study. There are a few calcified microconretions present in the lumen of renal tubules located at the corticomedullary junction and/or medulla of the kidney of some control and test animals which are attributed to naturally occurring disease. However, in three of the Group T-III animals (Nos. 25, 26 and 32) the renal concretions are unusually large and more numerous than those normally observed in untreated control dogs and are thought to be related to the experimental procedure.

There are no other changes that can be attributed to the test material or the test procedure.

D. E. Gordon

Donovan E. Gordon, D. V. M., Ph. D.
Diplomate, American College of
Veterinary Pathologists

TABLE XXVIII

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
1-M	Liver	-	-	Focal lymphoid infiltrations	+
	Lungs	-	-	Focal interstitial pneumonia	++
	Prostate	-	-	Focal chronic prostatitis	++
	Spleen	-	-	Hemosiderosis	+
2-M	Lungs	-	-	Chronic interstitial pneumonia	++
3-M	Heart	-	-	Congestion	+
	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Focal interstitial pneumonia	++
4-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
	Spleen	-	-	Hemosiderosis	+
5-F	Liver	-	-	Congestion	++
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
				Hyperemia	+

TABLE XXVIII continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
6-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Uterus	-	-	In estrus	-
7-F	Ovaries	-	-	Proestrus	-
	Liver	-	-	Congestion	++
8-F	Lungs	-	-	Chronic intersititial pneumonia	+

TABLE XXIX

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
9-M	Liver	-	-	Congestion	+
	Lungs	-	-	Hyperemia	+
10-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
11-M	Kidneys	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Congestion	+
				Focal lymphoid infiltration	+
12-M	Liver	-	-	Congestion	+
	Lung	-	-	Chronic interstitial pneumonia	++
13-F	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
14-F	Liver	-	-	Congestion	++
	Lungs	-	-	Bronchopneumonia	++

TABLE XXIX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
15-F	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
16-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++

TABLE XXX

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group II: 1.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
17-M	Lungs	-	-	Hyperemia	+
				Chronic interstitial pneumonia	++
18-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
19-M	-	-	-	-	-
20-M	Lungs	-	-	Chronic interstitial pneumonia	+
21-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
				Bronchopneumonia	++
22-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXX continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group II: 1.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
23-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Mesenteric lymph node	-	-	Hyperemia	+
	Pancreas	-	-	Hyperemia	+
24-F	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXXI

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
25-M	Liver	-	-	Congestion	+
	Kidney	-	-	Tubular concretions	+++
26-M	Liver	-	-	Congestion	+
	Kidney	-	-	Tubular concretions	+++
27-M	Liver	-	-	Congestion	+
	Lung	-	-	Chronic interstitial pneumonia	+
	Prostate	-	-	Chronic focal prostatitis	++
28-M	Kidneys	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	++
29-F	Liver	-	-	Congestion	++
	Lungs	-	-	Chronic interstitial pneumonia	++
30-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
31-F	Liver	-	-	Congestion	+

TABLE XXXI continued

TEST MATERIAL: Kasal

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
32-F	Gonads	-	-	Calcified follicle	+
	Kidneys	-	-	Tubular concretions	+++
	Liver	-	-	Focal lymphoid infiltration	+
	Spinal cord	-	-	Calcified debris in central canal	+

ALUMINIUM RETENTION AND TOXICITY IN CHRONIC RENAL FAILURE

H. THURSTON G. R. GILMORE
J. D. SWALES

Department of Medicine, Manchester University, and Universities' Research Reactor, Risley, Lancashire

Summary Aluminium hydroxide is extensively used to correct the hyperphosphatæmia of chronic renal failure, and it has been suggested that toxic amounts of aluminium are absorbed from this preparation. This hypothesis has been examined by administering aluminium hydroxide to normal growing rats and rats with chronic renal failure. Renal failure increased the deposition of aluminium in bone. Aluminium hydroxide impaired the growth-rate of normal rats and produced rachitic bone changes, but this effect could be corrected by phosphate supplements. No impairment of growth was produced by aluminium hydroxide in hyperphosphatæmic uræmic rats and no other pathological abnormalities could be demonstrated. Patients with chronic renal failure treated with aluminium hydroxide for prolonged periods showed similar bony levels of aluminium to the rats. The present work, whilst indicating that small quantities of aluminium are deposited in bone in chronic renal failure, indicates that aluminium hydroxide is non-toxic if hypophosphatæmia is avoided.

Introduction

PATIENTS with chronic renal failure usually develop hyperphosphatæmia, with a consequent danger of metastatic calcification.[1,2] Oral aluminium hydroxide ('Aludrox') is extensively used in such patients to lower the serum-phosphate level. Whilst it has been assumed that the aluminium so given is excreted in the fæces as insoluble aluminium phosphates, Berlyne et al.[3] found raised serum-aluminium levels in some patients to whom aluminium hydroxide had been administered, and they have suggested that aluminium is retained in renal failure.[4] Bailey et al.[5] found evidence for retention of very large quantities of aluminium in balance studies in chronic renal failure. On the other hand, the little that is known of aluminium toxicity suggests that it is attributable to phosphate depletion.[6]

The present investigation was conducted to see if aluminium deposition in the tissues is greater than normal when aluminium hydroxide is administered in chronic renal failure, and to assess the toxicity of this compound when hypophosphatæmia is prevented.

Materials and Methods

Groups of six weanling rats of the same mean weights were given (*a*) wholemeal diet; (*b*) wholemeal diet with aluminium hydroxide 3·2 g. per kg. added; (*c*) wholemeal diet with aluminium hydroxide plus 10 g. per kg. disodium hydrogen phosphate; or (*d*) prepared by three-quarters partial nephrectomy with a contralateral nephrectomy and given diet (*b*).

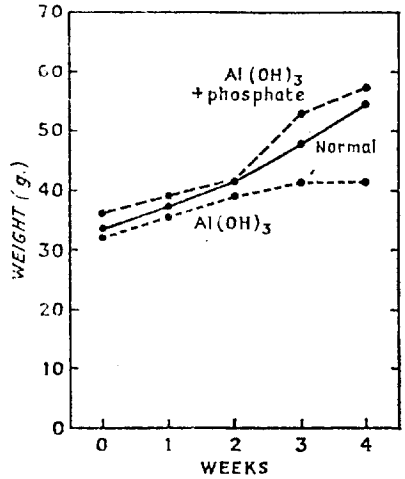
The animals were weighed weekly, and, after 4 weeks, were killed for blood-samples and complete post-mortem examination. Possible tissue contamination was avoided by removing the gut intact and unopened. Liver, kidney, and heart specimens were obtained for histology and analysis. The skeleton from each animal prepared by scraping was weighed after a tibia had been removed and a line test for rachitic change carried out. All the tissues were ashed at 400°C, dissolved in ion-exchange-purified 0·5*M* nitric acid, then run through an 'Amberlite' CG-120 (H^+) column to separate out the aluminium from the phosphate and calcium. The column was activated by thermal neutrons and the activity of aluminium-28 counted using a Ge (Li) detector. The activity of the column itself was subtracted using counts taken from a previous irradiation of the column. Measurement of standard solutions of aluminium nitrate by this technique gave a mean error of 4%.

Post-mortem iliac-crest bone specimens obtained from patients in advanced renal failure, with a known duration of aluminium-hydroxide treatment, were dried and ashed for aluminium determination by the same method.

Results

No animal died during aluminium-hydroxide administration.

Measurement of food intake indicated that rats consumed 6·0–10·0 mg. of aluminium per day, equivalent to a daily intake of 60 ml. of aluminium-hydroxide suspension by an adult 70 kg. man. Admini-



Growth of rats treated with aluminium hydroxide or aluminium hydroxide with supplemental phosphate compared with growth-rate in normal animals.

DR. REID AND OTHERS: REFERENCES

1. Allen, F. H., Diamond, L. K. Erythroblastosis Fetalis, including Exchange Transfusion. Boston, 1957.
2. McMullin, G. P., Hayes, M. F., Arora, S. C. *Lancet*, 1970, ii, 949.
3. Sisson, T. R. C., Glauser, S. C., Glauser, E. M., Tasman, W., Kuwabara, T. *J. Pediat.* 1970, **77**, 221.
4. Cremer, R. J., Perryman, P. W., Richards, D. H. *Lancet*, 1958, i, 1094.
5. Obes-Polleri, J. *Arches. Pediat. Urug.* 1967, **38**, 77.
6. Lucey, J. F., Ferriero, M., Hewitt, J. *Pediatrics, Springfield*, 1968, **41**, 1047.
7. Franklin, A. W. *Lancet*, 1958, i, 1227.
8. Hodgman, J. E., Teberg, A. *Birth Defects orig. Art. Ser.* 1970, **6**, 75.
9. Lucey, J. F. *ibid.* p. 63.
10. Broughton, P. M. G. *ibid.* p. 71.
11. Patel, D. A., Pildes, P. S., Behrman, R. E. *J. Pediat.* 1970, **77**, 1048.
12. Behrman, R. E., Hsia, D. Y. Y. *ibid.* 1969, **75**, 718.
13. *Br. med. J.* 1970, ii, 5.
14. Sisson, T. R. C., Kendall, N., Davies, R., Berger, D., Bunyaviroch, E. J., Knutson, S. *Pediat. Res.* 1969, **3**, 380.
15. Sisson, T. R. C., Kendall, N., Glauser, S. C., Knutson, S., Bunyaviroch, E. J. *J. Pediat.* 1971, **79**, 904.
16. Boggs, T. R., Hardy, J. B., Frazier, T. B. *ibid.* 1967, **71**, 553.

stration of aluminium hydroxide caused a significant impairment of growth at 3 and 4 weeks ($P<0.05$, see figure).

Animals receiving both aluminium hydroxide and the phosphate supplement showed a normal rate of growth (see figure). There was significant impairment of growth in uræmic rats but identical growth curves were obtained for aluminium-hydroxide-treated and untreated uræmic rats. Blood-urea levels in these animals ranged between 79 and 130 mg. per 100 ml. (mean 98 mg. per 100 ml.). Normal animals had blood-ureas ranging from 9 to 60 mg. per 100 ml. (mean 33 mg. per 100 ml.). Normal animals given aluminium hydroxide were hypophosphatæmic: the plasma-phosphate was normal or raised in the other groups (table I). Plasma-calcium was low in control animals (table I), reflecting the low calcium content of the diet. The line test indicated rachitic change in the normal rats treated with aluminium hydroxide. The line tests of the other animals were normal (table II). Histological examination of the tissues of all groups showed no abnormality. No eye lesions were found, although other animals in poor general state from the same colony not receiving aluminium hydroxide showed the hæmorrhagic eye lesions described by Berlyne et al.[4] These were attributed to infection.

Skeletal aluminium content was raised in the normal animals given aluminium hydroxide or aluminium hydroxide and phosphate, although the difference between these and the control groups was not statistically significant (table II). Uræmic animals showed significantly higher skeletal aluminium levels (table II). Pooled liver specimens showed a mean aluminium content of 78 μg. per 100 g. wet weight in the control rats; 37 μg. per 100 g. in the normal animals receiving aluminium hydroxide; and 66 μg. per 100 g. in uræmic rats given aluminium hydroxide. Corresponding figures for the hearts were 2·6 mg. per 100 g., 3·1 mg. per 100 g., and 2·5 mg. per 100 g. Aluminium content of the normal rats' kidneys was 4·1 mg. per 100 g. and the aluminium content of the aluminium-hydroxide treated animals was 4·2 mg. per 100 g. The total ashed weight of the heart and kidneys was, however, small, and the accuracy of the method is correspondingly less. The uræmic rats' kidneys were not studied because of the small amount of residual renal tissue. Patients showed bone-aluminium levels of the same order as the rats. The observed value was independent of the duration of aluminium-hydroxide therapy (table III).

TABLE I—PLASMA CALCIUM AND PHOSPHATE OF EXPERIMENTAL GROUPS

Group	Plasma-calcium (mg./100 ml.)	Plasma-phosphate (mg./100 ml.)
Control	5·4 ± 0·5	6·8 ± 0·5
Aluminium hydroxide	7·7 ± 1·0	2·1 ± 1·6
Aluminium hydroxide + phosphate	7·5 ± 0·6	7·3 ± 1·5
Uræmic rats: aluminium hydroxide	8·4 ± 1·3	6·8 ± 1·4

TABLE II—LINE TEST OF RACHITIC CHANGE AND SKELETAL ALUMINIUM CONTENT OF EXPERIMENTAL GROUPS

Group	Mean line-test score (normal = 5)	Aluminium content of skeleton	
		Mean ± S.E.M. (μg./100 g. wet weight)	Difference from controls
Control	5	471 ± 56	..
Aluminium hydroxide	2	717 ± 103	N.S.
Aluminium hydroxide + phosphate	5	943 ± 256	N.S.
Uræmic rats: aluminium hydroxide	5	1472 ± 346	$P < 0.05$

TABLE III—ALUMINIUM CONTENT OF ILIAC-CREST BONE IN PATIENTS DYING OF CHRONIC RENAL FAILURE AFTER ALUMINIUM-HYDROXIDE TREATMENT

Duration of aluminium-hydroxide therapy	Bone aluminium	
	(μg./100 g. wet weight)	(mg./kg. calcium)
Nil	1451	189
1 wk.	1273	155
5 mo.	910	103
7 mo.	920	212
16 mo.	735	97
19 mo.	939	147

Discussion

Aluminium is a common element found mainly in rocks rather than in soil or natural waters. Consequently only small quantities of aluminium are found in plants, and even less in animal tissues. It is believed to be poorly absorbed as a result of the formation of insoluble aluminium phosphate in the gastrointestinal tract. Chemical estimations indicate that animal tissues contain between 0·10 and 1·6 mg. of aluminium per 100 g. wet weight.[7] Chronic toxic effects of high doses of aluminium given orally seem to be associated with the disturbance of phosphate metabolism. These disturbances give rise to hypophosphatæmia, failure of growth, rickets,[8] low red-blood-cell A.T.P. levels, and reduced liver glycogen storage.[7]

Most patients with advanced renal failure are given large quantities of aluminium hydroxide without ill-effect. Occasional uræmic patients have developed a phosphate deficiency,[9] and one patient was shown to have reduced red-blood-cell A.T.P. activity which was correlated with serum-phosphate level.[10]

Parsons et al.[11] measured the aluminium content of biopsy and necropsy bone specimens in chronic renal failure. Some patients had large quantities of aluminium in the bone which, surprisingly, was not necessarily associated with aluminium-hydroxide therapy. These measurements were made on untreated bone ash by activation analysis, without allowance for the activation of phosphorus to aluminium-28 by the $^{31}P\ (n,\alpha)^{28}Al$ reaction.[12] The latter reaction, as judged by some of our early results using a similar method, can account for a variable increase in aluminium content of bone ash ranging from 1·5 to 10 times, compared with figures obtained after removal of phosphate by our method. Our results expressed as bony aluminium/calcium ratio are therefore about one-tenth of those found by Parsons et al.[11] (table III). Suggestions by Bailey et al.[5] that aluminium hydroxide lowers serum-phosphate by forming aluminium phosphate in bone would require an aluminium content of the order of 50–100 mg. per 100 g. wet weight, which is considerably greater than our results or even those of Parsons et al.[11]

Recent claims that aluminium is toxic to uræmic rats depend either upon the administration of soluble, astringent aluminium salts orally, or aluminium hydroxide by intraperitoneal injection.[4] The severe systemic effects and mortality produced by such materials in already sick animals cannot be extrapolated to the situation where insoluble aluminium hydroxide is administered orally to man, just as the demonstrable toxicity of barium chloride cannot be used to recommend the withdrawal of barium sulphate as a radiological medium. The pathological abnormalities described by Berlyne et al.[4] are frequently found in sick and uræmic animals, and no doubt the administration of soluble toxic aluminium salts exacerbated this picture.

Our results show that small quantities of aluminium are retained when aluminium hydroxide is administered in renal failure. There is no evidence from our experiments that these levels are toxic per se: thus, phosphate reversed the impairment of growth-rate without diminishing aluminium retention in bone. There was no macroscopic or microscopic abnormality in any of the other organs studied. Bone levels of aluminium in human patients treated for prolonged periods were of similar order to those encountered in our rat experiments, and it seems extremely unlikely therefore that other toxic effects occur in the human which cannot be demonstrated in rats. Recent evidence cannot justify the withdrawal of aluminium hydroxide as treatment for the hyperphosphatæmia of renal failure—still less has it any bearing on its widespread use as an insoluble antacid.

We thank Mr. Peter Chambers, Mrs. Jean Chadderton, Miss Ann Doyle, Mr. B. L. Goodwin, Miss Joyce Holland, and Miss Lynda Robison for the analyses. We are grateful to the Peel Trust for financial support.

Requests for reprints should be addressed to H. T., Department of Medicine, Royal Infirmary, Manchester M13 9WL.

REFERENCES

1. Stanbury, S. W. *in* Renal Disease (edited by D. A. K. Black); p. 537, Oxford, 1962.
2. Mallick, N. P., Berlyne, G. M. *Lancet*, 1968, ii, 1316.
3. Berlyne, G. M., Ben Ari, J., Pest, D., Weinberger, J., Stern, M., Gilmore, G. R., Levine, R. *ibid.* 1970, ii, 494.
4. Berlyne, G. M., Yagil, R., Ben Ari, J., Weinberger, G., Knopf, E., Danovitch, G. M. *ibid.* 1972, i, 564.
5. Bailey, R. R., Eastwood, J. B., Clarkson, E. M., Lucke, V. A., Hynson, W. V., O'Riordan, J. L. H., Woodhead, J. S., Clements, V., De Wardener, H. E. *Clin. Sci.* 1971, **41**, 5p.
6. Ondreička, R., Ginter, E., Kortus, J. *Br. J. ind. Med.* 1966, **23**, 305.
7. Ondreička, R., Kortus, J., Ginter, E. *in* Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides (edited by S. C. Skoryna and D. Waldron-Edward); p. 293. Oxford, 1971.
8. Jones, J. H. *Am. J. Physiol.* 1938, **124**, 230.
9. Dent, C. E., Harper, C. M., Philpot, G. R. *Q. Jl Med.* 1961, **30**, 1.
10. Lichtman, M. A., Miller, D. R., Freeman, R. B. *New Engl. J. Med.* 1969, **280**, 240.
11. Parsons, V., Davies, C. G., Goode, C., Ogg, C., Siddiqui, J. *Br. med. J.* 1971, iv, 273.
12. Goode, G. C., Howard, C. M., Wilson, A. R., Parsons, V. *Analytica chim. Acta*, 1972, **58**, 363.

EXCERPTS OF THE WORK OF THE STUDY COMMISSION ON FOREIGN SUBSTANCES IN FOODS

ALUMINUM IN FOODS

Report presented to the Study Commission on
"Foreign Substances in Foods"
by L. Truffert

The use of Aluminum for manufacturing utensils destined for food usage has been advocated by a number of authors and in particular, by my superior, E. Kohn-Abrest, from 1904, but it also has its detractors who have attributed the most varied disorders including cancer to the repeated ingestion of small amounts of Aluminum.

Normal Aluminum

From the work of Kahlenburg and Closs (1), Underhill, Peterman, Gross, and Krause (2), G. Bertrand and Mme. Levy (3), Yoshii and Jimbo (4), Wuhrer (5), Meunier (6) and (7), Webb (8), etc., it has been shown that Aluminum is normally present in all foods.

The figures shown by these authors differ somewhat, but it doesn't appear necessary to discuss their respective worth, because they differ rather little from those reported in the investigation relating to the examination of food substances (Minister of Agriculture and Supply, Central Bureau of Research, 1943). Let us note, however, that products of animal origin are much poorer in Aluminum than those of vegetable.

According to Calvery (9), the best estimation of the aluminum content of food substances includes between 10-30 mg/kg.

Contamination of Foods by Aluminum

The use of aluminum containers for foods is capable of introducing in them a supplementary amount of aluminum. This metal is very slightly attacked by pure water, with which on contact only a trace of aluminum, which is very slightly soluble (1.1×10^{-15} at 18°), is yielded, but this solubility increases considerably when it is in the presence of acidic or alkaline solutions. According to Gwyer and Pullen (10), almost neutral fresh milk does not at all dissolve a measurable fragment of aluminum, but from tests carried out in December 1943 by the Central Station of Microbiology applicable to Agricultural Industries of the Ministry of Agriculture, it was shown that the attack was not negligible, especially

when the milk products have been made acidic by the addition of 4 parts in 100 of lactic acid. These results have led A. Kling (11) to proscribe the use of aluminum for the manufacture of containers intended to contain liquid foods and most particularly milk products. Such a conclusion was too stringent as the use of aluminum in dairies was again recommended by E. Kohn-Abrest (12) and L. Truffert (13).

According to Turan (14), an acetic acid solution of 5 parts in 100, left 40 hours in an aluminum container dissolves only a trace of metal, whereas after heating it contains 20 mg. It is mainly acid or alkaline foods which react with the aluminum of vessels which contain them. Fruit preserves can contain up to 1.45 g/kg and their flavor is altered from 0.5 g of aluminum per kg.

However, G. Lunde (28) considers that aluminum boxes offer advantages of tin-plated iron boxes for the preservation of certain fish, especially sardines in oil. These aluminum boxes do not impart either any blackening or metallic taste; they are light and easy to open. Finally, the amount of dissolved aluminum is slight; from 5 to 64 mg per kg (for 2 to 24 months storage), whereas in boxes of tinned iron, the same author has found 24 to 527 mg of tin per kg. However, in boxes stored for many years, G. Lunde, V. Ascheboug and H. Kringstad (29) have found amounts of aluminum reaching around 100 mg per kg.

It is certain that using aluminum containers considerably increases the content of this metal in foods. According to Seppili (15), on the average it doubles the normal content.

The Fate of Ingested Aluminum

The question of the absorption of ingested aluminum has been highly debatable and it appears that the contradictory results are the consequence of the lack of sensitivity of some of the methods used to detect the aluminum.

Burn (16), Mackenzie (17), and Maas (18) have stated that all the ingested aluminum is recovered in practice in fecal matter and that only traces are possibly absorbed, traces too slight to be detected in the blood and the urine.

Wuhrer (5) claims that the whole ingested amount is quantitatively found in the feces when men and dogs are administered a diet containing up to 337 g of aluminum per day. One realizes that when working with such massive quantities of Aluminum it is not possible to ascertain whether traces of the order of a few milligrams have been absorbed by the organism.

Tourteloffe and Rask (19) in administering the very soluble compound, aluminum chloride, to the rat, likewise concluded that there wasn't any aluminum absorption.

On the other hand, Lewis (20) sometimes found aluminum in the blood after ingestion of this metal, while he regarded the blood plasma as being normally free of it.

Hove, Elvehljem and Hart (21) accept that a very small amount of aluminum is reabsorbed and eliminated by the urine. Petitpierre (22) regards this conclusion as the most reasonable.

It agrees with the results of Kehoe, Cholak, and Story (23), who have shown with a spectrographic study, the normal presence of Aluminum in the urine (of 0.02 to 0.17 mg/l) and in the blood (on an average 0.13 mg/l, of which the greater part is found in the plasma).

Moreover, let us note that the normal presence of aluminum in the tissues has been very controversial, but we do not here take up this discussion.

Here is the normal aluminum content of human organs, according to the spectrographic determinations of Kehoe, Chlolak, and Story (23).

	Aluminum in mg per kg of fresh organ
Brain	0.04
Lungs	59.4
Heart	0.56
Liver	1.6
Spleen	1.3
Kidney	0.42
Stomach	0.73
Intestines	0.87
Long bone	5.0
Ribs	2.4

These authors consider that the elevated aluminum content of the lungs is due to the inhalation of dust rich in this metal which is one of the most widespread minerals.

Calvery (9) thinks that, according to the work of Burn (16), of Monier-Williams (24), Kehoe, Cholak, and Story (25), that the amount of aluminum ingested daily would be about 13 mg. Beal, Unangst, Wigman and Cox (26) have calculated as 10 to 15 mg. the daily amount of aluminum consumed by an individual eating food cooked in saucepans made with this metal and calculated that about 5 mg to 12 mg comes from the vessels. Much larger amounts are given by Datta (27): 50 mg and by Turan (14): 110 mg. However, Turan and Burn agree to accept that about half of the daily amount they have shown comes from the utensils and the other half from the foods themselves.

Lunde, Aschenhoug and Keringstad (29) have determined aluminum in the organs of rats fed for several generations with food kept in aluminum. They obtained the following results by using precipitation with oxyquinoline or colorimetric determination with aurine tucarboxylate.

Aluminum in mg per kg in the organs of experimental rats absorbed from food preserved in Aluminum

Lungs	2	12	16	22	5
Heart	50	40	70	110	45
Liver	5	4	2	3	--
Spleen	55	40	50	30	--
Kidneys	6	15	11	12	--
Muscle	0	0	12	4	8

It does not appear that a sufficiently marked augmentation of the percentage of aluminum has been established to draw a conclusion. In fact, the spectrographic determinations of Kehoe, Cholak and Story (23) have shown that the normal aluminum content is very variable, especially in the blood and urine. This appears to indicate that this metal is not appreciably established in the organism and that the proportions found depend on the slight amount of ingested aluminum which can be momentarily absorbed, the largest amount being eliminated in the feces.

Nevertheless, certain authors like Underhill and Petermann (30 to 35), have claimed that ingested aluminum has the tendency to be deposited in the liver, the brain, the kidneys, the spleen and the thyroid in very small amounts, whereas the bile seems to be the most important pathway for

elimination. There appear to be serious reservations concerning these conclusions which do not appear to be supported by sufficiently convincing results. There are likewise some figures shown by Mull and his coworkers (36), on the subject of which, Kehoe, Cholak and Story (25), made the observation that the fact of finding ~~more~~ low values when the samples are very large indicates an important factor of contamination.

Toxicity of Aluminum

The method of administration of the aluminum has a considerable influence on its toxicity as has been shown by Bertrand and Sebescu (37) who, in experiments on the rabbit, have obtained the following results.

Subcutaneous injection of	Average Survival of
100 mg/kg	8 h 09
125 mg/kg	6 h 12
150 mg/kg	4 h 31
400 mg/kg	7 h 07

Aluminum appears therefore about four times less toxic by ingestion than by subcutaneous injection.

The same-authors (38) studied the toxicity of aluminum compared to that of other metals by means of the following procedure: They administered to guinea pigs by deep injection under the skin, 100 mg of metal per kg in aqueous sulfate solution of 1 to 1000 (except for Fe_{31} in the form of ferric chloride). The survival time of the animals (5 per metal) was observed and it was found:

		Survive an average of
Copper	--	0 h 52
	--	1 h
Cadmium	--	1 h 19
Cobal	--	2 h 17
Zinc	--	3 h 01
Manganese	--	7 h 14
Iron Trivalent	--	7 h 51
Aluminum	--	8 h 34
Iron Bivalent	--	8 h 44

The same was found for the rabbit

Copper	--	0 h 53
Nickel	--	1 h 11
Aluminum	--	8 h 09

This method seems hardly satisfactory to us, because it expresses rather than the toxicity itself the speed of the toxic action, which for some compounds, manifests itself by means of a long-term deadly effect at low doses. Nevertheless, in the case of aluminum it clearly seems that the toxicity is lower than that of the other metals studied.

Disturbances attributed to Aluminum

Some authors think that ingesting aluminum causes gastro-intestinal disturbances. Tocco (39) as well as Tchijevsky and Tchiveokaya (40) think that food which is improperly prepared in aluminum utensils can cause slight poisoning.

Putensen (41) has recorded cases of constipation with intestinal disturbances accompanied by black stools, owing to aluminum. He cites three cases of chronic poisoning from one family, caused by the use of aluminum containers and indicates the possibility of nervous difficulties with trembling as well as premature whitening of the hair.

Other authors have claimed that aluminum causes sterility or that it initiates the development of cancer. In this respect, Tchijevsky and Tchijevskaya (42) have reported that white mice fed with food prepared in aluminum plates became spontaneously cancerous in the proportion of 0.85 per 100 as compared with 0.02 per 100 for the controls.

Experiments on behalf of Aluminum

Tocco and Mulas (43) carried out research on the germination of seeds in the presence of powdered aluminum and on the fermentation of beer yeast without finding any noxious effect. They likewise performed toxicity tests on the frog and the rabbit by injecting them with large quantities of metallic aluminum or by dusting the rabbit food. They did not find any toxic effect with these animals.

Scott and Holz (44) administered food containing 3.6 parts per 100 of aluminum to rats for more than a year: Growth was perfect and the blood constitution remained normal.

Mackenzie (17) did not find any aluminum on several successive generations of rats.

Lunde and his coworkers (29) did not observe an effect on the growth of mice and have not observed any effect on rats for 5 generations.

Furthermore, Bertrand and Serbescu (45) using 116 rabbits treated with the object of initiating skin cancer, have established that ingesting aluminum does not increase the proportion of illness (on the contrary!).

Likewise, Baumann (46) was not able to increase the appearance of cancer by giving mice and rats aluminum salts or the powdered metal.

It appears then that the toxicity of aluminum has been greatly exaggerated, the symptoms attributed to it have not by any means been confirmed by the following.

Different Effects of Aluminum

Nevertheless one cannot knowingly say that aluminum does not sometimes have an effect on the organism, which has not been taken into account.

Kamarov and Krieger (47) have found that administering large amounts of the gel of aluminum hydrate (treatment for gastric ulcer) reduces the secretion of gastric juice, neutralizes its acidity and lowers peptic activity.

Kersner (48) gave men 393 cc of the same gel for 71 days, without finding disturbances of the acid-base equilibrium nor of kidney function.

However, absorption of such massive doses, if they are not toxic, inhibits the proper reabsorption of phosphorus contained in foods as has been shown in the work of Deobald and Elvehjem (49); Fauley and coworkers (50); Freeman (51); H. R. Street (52), etc. This disorder appears to be due to the formation of unsoluble aluminum in the digestive tract. It is harmful to growth and induces achitism, but is of less significance in adults. Moreover, iron salts have the same effect.

On the other hand, Schwab (53) brought attention to the effect of aluminum on certain hormones. This author has established that small amounts of aluminum chloride strengthen and prolong the hypoglycemic effect of insulin while strong doses inhibit the hormone. It is similar for the hyperglycemic effect of adrenalin.

Conclusions

It follows from this study that aluminum is not considered a toxic metal. It exists normally in all foods and using aluminum utensils for

preparing food is capable of doubling the amount normally ingested. Furthermore, one can sustain very large doses without harm, because in the U.S.A., they use a leavening agent based on an aluminum compound and from this fact that millions of people have swallowed this metal without experiencing the least harm. According to Kehoe, Cholak and Story (25) the daily ingestion of 200 mg of aluminum per day is without risk.

But still one cannot tolerate the presence of aluminum in foodstuffs in excessive amounts even if it does not represent any source of harm or does not alter the taste.

This is why it appears therefore logical to establish the maximum admissible content in foods, taking into account the much greater aluminum content of vegetable products as compared to those of animal origin:

Food Substances	Aluminum content (mg/kg)
1. Products of butchery, pork butchery, tripe shop, poultry, game	20 (fresh) and 100 (canned)
2. Fish	20 (fresh) and 100 (canned)
3. Molluses, crustacenas	29 (fresh) and 100 (canned)
4. Eggs	50
5. Oils and fats	50
6. Milk[1]	50
7. Wine, beer, cider, fruit juice and other alcholic or other beverages	50
8. Vegetables and fruits[2]	250
9. Cereals and derived products[2]	200
10. Cheeses[2]	200
11. Sugars, sweetening substances, jams[2]	200

[1] Dose expressed per liter.

[2] Content reported moisture free.

à distiller dès qu'on débouche le haut de la boule et qu'on arrête le gaz.

Il suffit donc de renouveler les quelques cc. de lessive de soude avant chaque distillation sans avoir besoin de vider et nettoyer la boule de neutralisation.

Nous avons pensé que la présentation de ce petit appareil pouvait intéresser ceux de nos collègues qui recherchent, en même temps que la précision, la rapidité dans la détermination du degré alcoolique des vins.

EXTRAITS DES TRAVAUX DE LA COMMISSION D'ETUDE DES SUBSTANCES ETRANGERES DANS LES ALIMENTS

L'ALUMINIUM DANS LES ALIMENTS

Rapport présenté à la Commission d'étude des « Substances étrangères dans les aliments »

par L. TRUFFERT

L'utilisation de l'aluminium pour la fabrication d'ustensiles destinés à des usages alimentaires a été préconisée par de nombreux auteurs et, en particulier, par mon maître, E. Kohn-Abrest, dès 1901, mais elle a eu aussi ses détracteurs qui ont attribué à l'ingestion répétée de petites quantités d'aluminium les troubles les plus divers, y compris le cancer.

Il est donc nécessaire d'étudier la teneur en aluminium des aliments avant et après séjour dans des récipients fabriqués avec ce métal, puis de chercher quel est le métabolisme de cet élément lorsqu'il est ingéré en proportions plus ou moins élevées.

Aluminium normal.

Il a été démontré que l'aluminium existait normalement dans tous les aliments, par les travaux de Kahlenberg et Closs (1), Underhill, Peterman, Gross et Krause (2), G. Bertrand et Mme Lévy (3), Yoshii et Jimbo (4), Wuhrer (5), Meunier (6) et (7), Webb (8), etc.

Les chiffres indiqués par ces auteurs diffèrent quelque peu, mais il ne semble pas nécessaire de discuter leurs valeurs respectives, car ils s'éloignent assez peu de ceux signalés dans l'Instruction relative à l'examen des matières alimentaires (Ministère de l'Agriculture et du Ravitaillement, Bureau Central des Recherches, 1943). Notons toutefois, que les produits d'origine animale paraissent beaucoup plus pauvres en aluminium que les végétaux.

D'après Calvery (9), la meilleure estimation de la teneur en aluminium des matières alimentaires serait comprise entre 10 et 30 mg./kg.

Pollution des aliments par l'aluminium

L'usage de récipients en aluminium pour les aliments est susceptible d'introduire dans ceux-ci une quantité supplémentaire d'aluminium. Ce métal est très peu attaqué par l'eau pure au contact de laquelle il donne des traces d'alumine dont la solubilité est extrêmement faible ($1{,}1.10^{-15}$ à 18°), mais cette solubilité augmente considérablement lorsqu'on se trouve en présence de solutions alcalines ou acides. D'après Gwyer et Pullen (10), le lait frais presque neutre ne dissoudrait aucune parcelle appréciable d'aluminium, mais des essais effectués en décembre 1943 par la Station Centrale de Microbiologie appliquée aux Industries agricoles du Ministère de l'Agriculture, ont montré que l'attaque n'était pas négligeable, surtout lorsque les produits laitiers étaient rendus acides par addition de 1 pour 100 d'acide lactique. Ces résultats avaient amené A. Kling (11) à proscrire l'emploi de l'aluminium pour la fabrication des récipients destinés à contenir des liquides alimentaires et plus particulièrement des produits laitiers. Une telle conclusion était trop sévère, aussi l'usage de l'aluminium en laiterie a-t-il été préconisé à nouveau par E. Kohn-Abrest (12) et L. Truffert (13).

D'après Turan (14), une solution d'acide acétique à 0.5 pour 100, laissée 40 heures dans un récipient en aluminium ne dissout que des traces de métal, alors qu'après cuisson elle en renferme 20 mg. Ce sont surtout les aliments acides ou alcalins qui réagissent avec l'aluminium des récipients qui les renferment. Les conserves de viande peuvent en contenir jusqu'à 1,45 g./kg. et leur goût est altéré à partir de 0,5 g. d'aluminium par kg.

Cependant G. Lunde (28) estime que les boîtes d'aluminium présentent des avantages sur les boîtes de fer étamé pour la conservation de certains poissons, en particulier les sardines à l'huile. Ces boîtes en aluminium ne donnent aucun noircissement, ni goût métallique ; elles sont légères et faciles à ouvrir. Enfin les quantités d'aluminium dissoutes sont faibles : de 5 à 61 mg. par kg. (pour une conservation de 2 à 21 mois), alors que dans les boîtes en fer blanc, le même auteur a trouvé de 21 à 527 mg. d'étain par kg. Toutefois, dans les boîtes conservées pendant plusieurs années, G. Lunde, V. Aschehoug et H. Krinsgstad (29) ont trouvé des quantités d'aluminium atteignant environ 100 mg. par kg.

Il est certain que l'emploi de récipients en aluminium augmente notablement la teneur de ce métal dans les aliments. D'après Seppili (15), il doublerait en moyenne la teneur normale.

Sort de l'aluminium ingéré

La question de l'absorption de l'aluminium ingéré a été très discutée et il semble que les résultats contradictoires soient

la conséquence du manque de sensibilité de certaines des méthodes utilisées pour déceler l'aluminium.

Burn (16), Mackenzie (17) et Maas (18) ont affirmé que tout l'aluminium ingéré se retrouvait pratiquement dans les matières fécales et que seules des traces étaient peut-être absorbées, traces trop faibles pour être décelées dans le sang et dans l'urine.

Wuhrer (5) prétend que toute la quantité ingérée se retrouve quantitativement dans les matières fécales lorsqu'on administre à des hommes et à des chiens une nourriture contenant jusqu'à 337 g. d'aluminium par jour. On comprend qu'en opérant sur des quantités aussi massives d'aluminium il n'ait pu se rendre compte si des traces de l'ordre de quelques milligrammes étaient absorbées par l'organisme.

Tourtelotte et Rask (19), en administrant au rat du chlorure d'aluminium, corps très soluble, conclurent également qu'il n'y avait aucune absorption d'aluminium.

Par contre, Lewis (20) trouva parfois de l'aluminium dans le sang après ingestion de ce métal, alors qu'il considérait le plasma sanguin comme en étant exempt normalement.

Hove, Elvehjem et Hart (21) admettent qu'une très petite quantité d'aluminium est résorbée et éliminée par les urines. Petitpierre (22) estime que c'est la conclusion la plus raisonnable.

Elle s'accorde avec les résultats de Kehoe, Cholak et Story (23), qui ont montré, par une étude spectrographique, la présence normale d'aluminium dans l'urine (de 0,02 à 0,17 mg./l.) et dans le sang (en moyenne 0,13 mg./l., dont la majeure partie se trouverait dans le plasma).

Notons d'ailleurs que la présence normale de l'aluminium dans les tissus a été très controversée, mais nous n'aborderons pas ici cette discussion.

Voici, d'après les déterminations spectrographiques de Kehoe, Cholak et Story (23), la teneur normale en aluminium des organes humains :

	Aluminium en mg. par kilog d'organe frais
Cerveau	0,04
Poumons	59,4
Cœur	0,56
Foie	1,6
Rate	1,3
Reins	0,42
Estomac	0,73
Intestins	0,87
Os long	5,0
Côtes	2,4

Ces auteurs estiment que la teneur élevée en aluminium des poumons est due à l'inhalation des poussières, riches en ce métal qui est l'un des constituants des minéraux les plus répandus.

Calvery (9) estime, d'après les travaux de Burn (16), de Monier-Williams (24), Kehoe, Cholak et Story (25), que la quantité d'aluminium ingérée journellement serait d'environ 13 mg. Beal, Unangst, Wigman et Cox (26) avaient évalué de 10 à 15 mg. la dose journalière d'aluminium consommée par un individu mangeant de la nourriture cuite dans des casseroles faites avec ce métal et estimaient qu'environ 5 mg. sur 12 mg. provenaient des récipients. Des doses plus considérables étaient indiquées par Datta (27) : 50 mg., et par Turan (14) : 110 mg. Toutefois, Turan et Burn s'accordent pour admettre qu'environ la moitié des doses journalières qu'ils indiquent provient des ustensiles de cuisine et l'autre moitié des aliments eux-mêmes.

Lunde, Aschehoug et Kringstad (29) ont dosé l'aluminium dans les organes de rats nourris depuis plusieurs générations avec des aliments conservés dans l'aluminium. Ils ont obtenu, en utilisant la précipitation par l'oxyquinoléine ou la détermination colorimétrique à l'aurine-tricarboxylate, les résultats suivants :

	Aluminium en mg. par kg. dans les organes de rats témoins absorbant de la nourriture conservée dans de l'aluminium				
Poumons	2	12	16	22	5
Cœur	50	40	70	110	45
Foie	5	3	2	3	—
Rate	55	40	50	30	—
Reins	6	15	11	12	—
Muscle	0	0	12	4	8

Il ne semble pas que l'on constate une augmentation suffisamment marquée de la proportion d'aluminium pour en tirer une conclusion. En effet, les déterminations spectrographiques de Kehoe, Cholak et Story (23) ont montré que les teneurs en aluminium normal étaient très variables, en particulier pour le sang et l'urine. Ceci paraît indiquer que ce métal n'est notablement pas fixé dans l'organisme et que les proportions trouvées dépendent de la faible portion de l'aluminium ingéré qui a pu être momentanément absorbée, la plus grande partie étant éliminée dans les excréments.

Cependant, certains auteurs, comme Underhill et Petermann (30 à 35), ont prétendu que l'aluminium ingéré avait tendance à se déposer dans le foie, le cerveau, les reins, la rate et la thyroïde en très petites quantités, alors que la bile sem-

blait la voie d'élimination la plus importante. Il convient de faire de sérieuses réserves concernant ces conclusions qui ne paraissent pas étayées sur des résultats suffisamment probants. Il en est de même des chiffres indiqués par Mull et ses collaborateurs (36), au sujet desquels, Kehoe, Cholak et Story (25) font remarquer que le fait de trouver des valeurs plus basses lorsque les échantillons sont plus forts, indique un important facteur de contamination.

Toxicité de l'aluminium

Le mode d'administration de l'aluminium influe considérablement sur sa toxicité comme l'ont démontré Bertrand et Serbescu (37) qui, en expérimentant sur le lapin, ont obtenu les résultats suivants :

Injection sous-cutanée de	Survie moyenne de
100 mg./kg.	8 h. 09
125 mg./kg.	6 h. 12
150 mg./kg.	4 h. 31
Ingestion de 400 mg./kg.	7 h. 07

L'aluminium paraît donc environ quatre fois moins toxique par ingestion que par injection sous-cutanée.

Les mêmes auteurs (38) avaient étudié la toxicité de l'aluminium, comparée à celle d'autres métaux en opérant de la manière suivante : on administre à des cobayes 100 mg. de métal par kg., en injectant profondément sous la peau des solutions aqueuses de sulfates à 1 pour 1.000 (sauf pour Fe+++, sous forme de chlorure ferrique). On note le temps de survie des animaux (5 par métal) et l'on trouve :

Cuivre	Survie moyenne de	9 h. 52
Nickel	—	1 h.
Cadmium	—	1 h. 19
Cobalt	—	2 h. 17
Zinc	—	3 h. 01
Manganèse	—	7 h. 14
Fer trivalent	—	7 h. 51
Aluminium	—	8 h. 34
Fer bivalent	—	8 h. 44
Sur le lapin, on trouve de même :		
Cuivre	—	0 h. 53
Nickel	—	1 h. 11
Aluminium	—	8 h. 09

Cette méthode ne nous paraît guère satisfaisante, car elle exprime plutôt la rapidité de l'action toxique que la toxicité elle-même qui, pour certains corps, se manifeste par une action mortelle à longue échéance avec de très faibles doses. Toutefois, dans le cas de l'aluminium, il semble bien que la toxicité soit moindre que celle des autres métaux étudiés.

Troubles imputés a l'aluminium

Certains auteurs estiment que l'ingestion d'aluminium provoque des troubles gastro-intestinaux. Tocco (39), ainsi que Tchijevsky et Tchijevskaya (10) estiment qu'une nourriture mal préparée dans des ustensiles en aluminium peut causer de légers empoisonnements.

Putensen (11) a constaté des cas de constipation avec troubles intestinaux accompagnés de selles noires, dûs à l'aluminium. Il cite trois cas d'intoxication chronique, dont un familial, causés par l'usage de récipients en aluminium et indique la possibilité de troubles nerveux, avec tremblements ainsi qu'un blanchiment prématuré des cheveux.

D'autres auteurs ont prétendu que l'aluminium provoquait la stérilité ou qu'il suscitait le développement du cancer. A cet égard, Tchijevsky et Tchijevskaya (42) ont rapporté que des souris blanches nourries avec des aliments préparés dans des plats d'aluminium devenaient cancéreuses spontanément dans la proportion de 0,85 pour 100 au lieu de 0,02 pour 100 chez les témoins.

Expérimentations en faveur de l'aluminium

Tocco et Mulas (43) ont exécuté des recherches sur la germination des semences en présence de poudre d'aluminium et sur la fermentation avec la levure de bière, sans trouver aucune action nocive. Ils firent également des essais d'intoxication sur la grenouille et le lapin en leur injectant de grandes quantités d'aluminium métallique ou en saupoudrant la nourriture des lapins. Ils n'ont trouvé aucune action toxique sur ces animaux.

Scott et Holz (14) ont administré à des rats une nourriture renfermant 3,6 pour 100 d'aluminium pendant plus d'une année : la croissance a été parfaite et la formule sanguine est restée normale.

Mackenzie (17) n'a constaté aucun effet de l'aluminium sur plusieurs générations successives de rats.

Lunde et ses collaborateurs (29) n'ont pas observé d'action sur la croissance de la souris et n'ont noté aucun effet sur les rats durant cinq générations.

D'autre part, Bertrand et Serbescu (45), utilisant 116 lapins traités en vue de provoquer des cancers cutanés, ont constaté

que l'ingestion d'aluminium n'augmentait pas la proportion des malades (au contraire !).

De même, BAUMANN (46) n'avait pu favoriser l'apparition du cancer en donnant à des souris et à des rats des sels d'aluminium ou ce métal en poudre.

Il semble donc que l'on ait grandement exagéré la nocivité de l'aluminium, les accidents lui ayant été imputés ne s'étant nullement confirmés par la suite.

ACTIONS DIVERSES DE L'ALUMINIUM

On ne saurait dire cependant que l'aluminium n'a pas parfois une action sur l'organisme, dont il faut tenir compte.

KOMAROV et KRUEGER (17) ont trouvé que l'administration de grosses quantités de gèl d'hydrate d'aluminium (traitement de l'ulcère gastrique) diminuait la sécrétion du suc stomacal, neutralisait son acidité et abaissait l'activité peptique.

KIRSNER (48) a administré à des hommes 393 cm³ d'un tel gèl pendant 71 jours, sans constater de troubles de l'équilibre acido-basique ni de la fonction rénale.

Toutefois, l'absorption de telles doses massives, si elle n'est pas toxique, inhibe la bonne résorbtion du phosphore contenu dans les aliments ainsi que l'ont montré les travaux de DEOBALD et ELVEHJEM (19), FAULEY et ses collaborateurs (50), FREEMAN (51), H. R. STREET (52), etc. Ce trouble paraît dû à la formation, dans le tube digestif, d'aluminium insoluble. Il peut nuire à la croissance et provoquer du rachitisme, mais présente moins d'importance chez l'adulte. Les sels de fer auraient d'ailleurs le même effet.

D'autre part, l'action de l'aluminium sur certaines hormones a été mise en évidence par SCHWAB (53). Cet auteur a constaté que de faibles quantités de chlorures d'aluminium renforçaient et prolongeaient l'action hypoglycémiante de l'insuline alors que de fortes doses inhibaient l'hormone. Il en est de même pour l'action hyperglycémiante de l'adrénaline.

CONCLUSIONS

Il ressort de cette étude que l'aluminium n'est pas à considérer comme un métal toxique. Il existe normalement dans tous les aliments et l'usage d'ustensiles en aluminium pour la préparation de la nourriture est susceptible de doubler la quantité normalement ingérée. On peut d'ailleurs, sans inconvénient, supporter des doses beaucoup plus considérables, car aux U.S.A., on a utilisé un levain à base de composés d'aluminium et, de ce fait, des millions de personnes ont avalé, chaque jour, des proportions importantes de ce métal sans en ressentir le moindre inconvénient. D'après KEHOE, CHOLAK et STORY (25), l'ingestion journalière de 200 mg. d'aluminium par jour serait sans danger.

Mais, cependant, on ne saurait tolérer la présence de proportions excessives d'aluminium dans les matières alimentaires, même si elle ne constitue aucune source de danger et ne dénature pas le goût.

C'est pourquoi, il semble logique de fixer ainsi les teneurs maxima admissibles dans les aliments, en tenant compte de la plus grande richesse en aluminium des produits végétaux, par rapport à ceux d'origine animale :

Substances alimentaires	Teneur en aluminium (mgr. par kilog)
1° Produits de boucherie, charcuterie, triperie, volailles, gibier	20 (frais) et 100 (en conserve)
2° Poissons	20 (frais) et 100 (en conserve)
3° Mollusques, crustacés	20 (frais) et 100 (en conserve)
4° Œufs	50
5° Huiles et graisses	50
6° Lait (1)	50
7° Vin, bière, cidre, jus de fruits et autres boissons alcoolisées ou non	50
8° Légumes et fruits (2)	250
9° Céréales et produits dérivés (2)	200
10° Fromages (2)	200
11° Sucres, matières sucrées, confitures (2)	200

(1) Dose exprimée par litre.
(2) Teneur rapportée à la manière sèche.

Paris, le 17 juin 1949.

BIBLIOGRAPHIE

(1) L. KAHLENBERG et J. O. CLOSS. — La présence d'aluminium dans les matières animales et végétales. J. Biol. Chem., t. 83, p. 261 (1929).

(2) F. P. UNDERHILL, F. I. PETERMAN, E. G. GROSS et A. C. KRAUSE. — Le métabolisme de l'aluminium VII. Teneur en aluminium de quelques aliments frais. Am. J. Physiol., t. 90, p. 72 (1929).

(3) BERTRAND G. et Mme G. LÉVY. — La teneur des plantes, notamment des plantes alimentaires en aluminium. C. R. Ac. Sc., t. 192, p. 525-529 (mars 1931).

(4) Y. YOSHII et T. JIMBO. — Détection microchilique de l'aluminium et sa présence dans le règne végétal. Science Repts. Tôhoku Imp. Univ. Fourth Ser., t. 7, p. 85 (1932).

(5) J. WUHRER. — L'absorption des composés de l'aluminium dans l'organisme et examen de la teneur normale en aluminium des tissus animaux. Biochem. Z., t. 265, p. 169 (1933).

(6) P. MEUNIER. — Nouvelle méthode de dosage de très petites quantités d'aluminium dans les milieux complexes. Application aux végétaux. C. R. Ac. Sc., t. 199, p. 1250-1252 (1934).

(7) P. MEUNIER. — Sur la présence et la répartition de l'aluminium dans les tissus animaux. C. R. Ac. Sc., t. 203, p. 891-894 (1936).

(8) D. A WEBB. — Etudes sur la composition ultime des matières biologiques II. Analyses spectrographiques des invertébrés marins et rapport avec la composition chimique de leur milieu. Sci. Proc. Roy. Soc. (Dublin), t. 21, p. 505 (1937).

(9) H. O. CALVERY. — Eléments, traces dans les aliments. Food. Research, t. 7, p. 313-336 (1942).

(10) A G. C. GWYER et N. D. PULLEN. — Analyst, t. 57, p. 704 (1932).

(11) A. KLING. — Rapport au Conseil Supérieur d'Hygiène Publique de France (19 février 1945).

(12) E. KOHN-ABREST. — Avantages de l'emploi de l'aluminium pour le transport et la manutention du lait. Société des Experts-Chimistes, séance du 8 décembre 1948.

(13) L. TRUFFERT. — Rapport au Conseil Supérieur d'Hygiène Publique de France (8 novembre 1948).

(14) G. TURAN. — Aluminium, t. 12, p. 3 (1930).

(15) SEPPILI. — Igiène Mod., t. 27, p 1 (1934).

(16) J. H. BURN. — L'action physiologique de l'aluminium. Analyst., t. 57, p. 428-440 (1932).

(17) K. MACKENZIE. — Biochem. J., t. 26, p. 833 (1932).

(18) T. A. MAAS. — Z. für Frnährg, t. 1, p. 193 (1931).

(19) D. TOURTELOTTE et O. S. RASK. — Amer. J. Hyg., t. 14, p. 225 (1931-32).

(20) S. J. LEWIS. — Biochem. J., t. 25, p. 2162 (1931).

(21) E. HOVE, C. A. EVEHIJEM et E. B. HART. — Amer. J. Physiol., t. 123, p. 640 (1938).

(22) CL. PETITPIERRE. — Gesundheit u. Wohlfahrt, p. 181-189.

(23) R. A. KEHOE, J. CHOLAK et R. V. STORY. — Etude spectrochimique de la teneur normale de certains oligoéléments dans les matières biologiques. J. Nutrit., t. 19, p 579-592 (janvier-juin 1940).

(24) G. W. MONIER-WILLIAMS. — L'aluminium des aliments. Rep. Pub. Health and Med. Subj. n° 78 (Great Britain, 1935).

(25) R. A. KEHOE, J. CHOLAK et R. V. STORY. — Manganèse, plomb, étain, aluminium, cuivre et argent dans les matières biologiques normales. J. Nutrit., t. 20, p. 85 (1940).

(26) G. D. BEAL, R. B. UNANGST, H. B. WIGMAN et G. J. COX. — Ind. Eng. Chem., t. 24, p. 405 (1923).

(27) N. C. DATTA. — Proc. Indian Acad. Sci., t. 2, p. 322 (1935).

(28) G. LUNDE. — Tidsskrift for Hermetikindustri, t. 18, p. 205-216 (1932).

(29) G. LUNDE, V. HASCHEHOUG et H. KRINGSTAD. — J. Soc. Chem. Ind., t. 56, p. 334-339 (septembre 1937).

(30) F. P. UNDERHILL et F. I. PETERMAN. — Etudes sur le métabolisme de l'aluminium. II. Absorption et fixation de l'aluminium chez le chien. Am. J. Physiol., t. 90, p. 15 (1929).

(31) F. P. UNDERHILL et F. I. PETERMAN. — III. Absorption et excrétion de l'aluminium chez l'homme normal. Am. J. Physiol., t. 90, p. 40 (1929).

(32) F. P. UNDERHILL, F. I. PETERMAN, E. G. GROSS et A. C. KRAUSE. — L'aluminium dans le foie et le rein humains. Am. J. Physiol., t. 90, p. 90, p 67 (1929).

(33) F. P. UNDERHILL, F. I. PETERMAN, E. G. GROSS et A. C. KRAUSE. — La teneur en aluminium de quelques aliments frais. Am. J. Physiol., t. 90, p. 72 (1929).

(34) UNDERHILL, PETERMAN et SPERANDEO. — Effet toxique de l'aluminium par injection sous-cutanée. Am J. Physiol., t. 90, p. 76 (1929).

(35) UNDERHILL, PETERMAN et STELL. — Métabolisme de l'aluminium introduit par injection intraveineuse. Am. J. Physiol., t. 90, p. 52 (1929).

(36) J. W. MULL, D. B. MORRISON et V. C. MYERS. — La présence d'aluminium dans le sang. Proc. Soc Exptl. Biol. Med., t. 24, p. 476 (1927).

(37) G. BERTRAND et P. SERBESCU. — La toxicité de l'aluminium selon la voie d'entrée. C. R. A. Sc., t. 198, p. 517-520 (1934).

(38) G. BERTRAND et P. SERBESCU. — Sur la toxicité de l'aluminium, comparée à celle du fer, du nickel et d'autres métaux. C. R Ac. Sc., t. 193, p. 129-131 (1931).

(39) L. TOCCO. — Boll. Soc. Ital. Biol. sper., t. 20, p. 65 (1935).

(40) A. L. TCHIJEVSKY et T. S. TCHIJEVSKAYA. — Acta Med. Scandin., t. 83, p. 501 (1934).

(41) POTENSEN. — Aluminium, t. 12, n°s 15-16, p. 4 (1930).

(42) A. L. TCHIJEVSKY et T. S. TCHIJEVSKAYA. — Gaz des Hôpitaux, 1934, n° 47, p. 853.

(43) L. TOCCO et M. MULAS. — Arch. Intern. Pharmacodyn. Therap., t. 51, fasc. 2, p. 159-170 (1935).

(44) E. SCOTT et M. K. HOLZ. — Amer. J. Hyg., t. 16, p. 865 (1932).

(45) G. BERTRAND et P. SERBESCU. — L'ingestion journalière de petites quantités d'aluminium favorise-t-elle le cancer ? C. R Ac. Sc., t. 198, p. 1100-1103 (1934).

(46) O. BAUMANN. — Chem. Z., t. 57, p. 424 (1933).

(47) S. A. KOMAROV et L. KRUEGER. — Amer. J. Digest. Dis., t. 7, p 170 (1940).

(48) J. B. KIRSNER. — Amer. J. Digest. Dis., t. 8, p. 160 (1941).

(49) H. J. DEOBALD et C. A. ELVEHJEM. — Amer. J. Physiol., t. 111, p. 118 (1935)

(50) G. B. FAULEY, S. FREEMAN, A. C. IVY, A. J. ATKINSON et H. S. WILDFANG. — Arch. Intern. Méd., t. 67, p. 563 (1941).

(51) S. et W. M C. FREEMAN. — Amer. J. Dis. Childr., t. 61, p. 981 (1941).

(52) H. R. STEET. — J. Nutrit., t. 24, p. 111 (1942).

(53) H. SCHWAB. — C. R. Ac. Sc., t. 206, p. 211 (1938).

LE CHROME DANS LES ALIMENTS

Rapport présenté à la Commission d'étude des « Substances étrangères dans les aliments »

par Louis TRUFFERT

Le chrome est un métal que l'on rencontre rarement en proportions appréciables dans les aliments et sur la toxicité duquel des opinions contradictoires se sont affrontées, sans

Am J Physiol 90: 15-27 (1929)

STUDIES IN THE METABOLISM OF ALUMINIUM

II. Absorption and Deposition of Aluminium in the Dog[1]

FRANK P. UNDERHILL AND F. I. PETERMAN

From the Department of Pharmacology and Toxicology, Yale University, New Haven, Conn.

Received for publication April 19, 1929

The fact that the majority of investigators have not found aluminium in the blood and tissues of animals after a diet containing appreciable amounts of it (1), has in the main been attributed to the formation, in the gastro-intestinal tract, of insoluble compounds, that is, aluminium hydroxide, or phosphates, or aluminium-protein complexes. That insoluble compounds may be formed during the baking of aluminium-containing breads has also been suggested.

It has been demonstrated that some of the aluminium occurring naturally in foods is soluble in aqueous solutions (2). It has been shown repeatedly that a large percentage of the aluminium in bread made with alum baking powders is soluble in gastric juice (1). It is unknown whether aluminium forms some complex protein compound in bread. At any rate after exposure to gastric juice, either *in vitro* or *in vivo*, the resulting compound is dialyzable, and is presumably the chloride.

Information as to the exact degree of alkalinity of the intestinal tract is lacking but the available evidence seems to indicate that the pH does not vary widely from pH 7.0 (3) (4) (5).

The pH for the precipitation of aluminium hydroxide varies, of course, with the constituents of the solution. In pure solutions it begins to precipitate at pH 4.1 (6). But the hydroxide is truly amphoteric, the soluble aluminates forming promptly. Aluminium phosphate begins to precipitate, under various conditions at pH 3.9 to 5.5 (7) (8) (9). It starts to redissolve at pH 8.6.

While it is injudicious to hazard a guess as to what happens in an exceedingly complex mixture it seems probable that aluminium compounds may be largely soluble in the intestinal tract.

[1] Constructed, in part, from a dissertation presented to Yale University in June, 1925, by F. I. Peterman in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Summarizing we may suppose that aluminium occurring naturally in foods, or added for leavening purposes, is soluble to a certain extent in both stomach and intestinal tract. Since it is soluble, we must expect that it will be absorbed into the body.

The purpose of the present investigation was to obtain information relative to the following problems:

1. The aluminium content of the blood of dogs
   - *a*, in the normal fasting condition
   - *b*, on a diet to which the aluminium has been added
   - *c*, at various times after a single feeding of food to which aluminium has been added
   - *d*, during and after a dietary of aluminium containing foods for periods of
     - (1) 1 week
     - (2) 4 weeks
     - (3) 12 weeks
   - *e*, during and after an ordinary dietary for periods corresponding to *d*
2. The aluminium content of the tissues of these dogs.

General methods. The experimental animals, in good nutritive condition, were of unknown origin but were full grown and as near as one may judge did not exceed five years in age. Kept in separate cages in the animal house they were fed ground lean beef, aluminium-containing biscuits and butter. Control animals were fed yeast bread instead of biscuits. The dogs were given as much as they would eat, but only one feeding per day.

Two kinds of baking powder were used in the preparation of the biscuits, which for the sake of brevity will be designated as "straight alum" and "alum-phosphate" biscuits.

As it was not possible to purchase a straight alum baking powder on the local market one was prepared in the laboratory, and had the following composition:

Sodium aluminium sulphate, calcined	25 grams
Sodium bicarbonate, dry	26 grams
Corn starch	49 grams

This powder was used in biscuits as follows:

Flour	8100 grams
Salt	75 grams
Baking powder	540 grams
Butter	300 grams
Water	

The "alum-phosphate" powder used was a well-known brand, bought on the market, and according to the label its composition was:

Mono calcium phosphate	15.67 per cent
Sodium aluminium sulphate	12.33 per cent
Sodium bicarbonate	25.00 per cent
Starch	47.00 per cent

TABLE 1

The aluminium content of blood of fasting dogs

Al as mgm. per 100 cc. of blood

DAY	DOG 1	DOG 2	DOG 3	DOG 4	DOG 5	DOG 6	AVERAGE
1	0.28	0.35	0.28	0.32	0.17	0.17	0.23
3	0.29	0.32	0.28	0.27	0.17	0.17	
5	0.28	0.28	0.27	0.17	0.16	0.18	
7	0.28	0.27	0.27	0.16	0.16	0.16	
Average....	0.28	0.30	0.27	0.23	0.16	0.17	

TABLE 1A

Aluminium content of tissues of dogs fasted one week

Al as mgm. per 100 grams of wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
1	17.3	F.	0.66	1.80	0.57	1.22	0.138	0.213	1.36	1.01	None		5.5
2	16.0	M.	0.69	2.01	0.74	1.10	0.135	0.222	1.41	None		None	6.0
3	18.6	M.	0.89	1.85	0.84	1.05	0.135	0.191	2.06	2.49		None	7.4
4	12.0	F.	0.80	2.84	0.94	1.08	0.200	None	2.04	None	None		11.4
5	15.5	M.	0.90	1.39	0.66	1.27	0.131	None	1.67	None		. —	4.7
6	14.0	F.	0.71	2.16	0.56	1.17	None	0.937	2.27	None	None		5.0
7	16.4	F.	0.90	2.99	1.45	0.96	0.125	0.270	2.29	1.69	None		8.2
8	14.8	F.	0.75	1.74	0.90	1.18	0.142	None	1.43	None	None		8.2
9	3.6	M.	1.41	1.00	0.84	1.31	0.127	0.252	2.37	None		Trace	10.3
10	8.9	M.	0.95	2.19	1.20	1.21	0.129	Trace	2.38	None		Trace	12.4
11	9.0	F.	1.33	0.92	0.89	2.17	None	None	1.32	1.01	None		6.1
12	15.0	M.	1.16		0.72			0.220	1.49	None		None	2.3
Average....			0.94	1.90	0.86	1.25	0.114	0.192	1.84	0.51	None	0.000	7.25

Biscuits were made approximately according to the directions on the can, and contained:

Flour	8750 grams
Ba[illegible]owder	500 grams
S[illegible]	80 grams
B[illegible]	300 grams

The biscuit dough was mixed and baked much as it is by the housewife. After baking it was broken into pieces, thoroughly dried and then ground to a coarse meal. The dogs would eat a moist mixture of hashed meat and biscuit meal, but if the biscuit were given in pieces they would eat the meat and leave the biscuit. Biscuit dried and ground kept well without

TABLE 2

Aluminium content of blood of dogs fed on control diet for one week

Al as mgm. per 100 cc. of blood

DAY	DOG 19	DOG 20	DOG 21	DOG 22	DOG 23	DOG 24	AVERAGE
1		0.45	0.37	0.31	0.29	0.27	0.36
5	0.35	0.48	0.44	0.31	0.38	0.30	
8	0.36	0.48	0.40	0.37	0.37	0.31	
Average....	0.35	0.47	0.40	0.33	0.34	0.29	

TABLE 2A

Aluminium content of tissues of dogs after one week's feeding of "normal diet"

Al as mgm. per 100 grams of wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
13	10.6	F.	0.77	—	1.56	1.64	—	None	2.39	None	None		2.7
14	14.0	F.	0.56	1.56	1.40	1.95	None	0.227	2.09	None	None		8.5
15	7.4	F.	0.93	1.62	1.40	2.14	None	Trace	2.03	None	—		7.4
16	5.6	F.	0.74	2.77	0.96	1.33	None	0.513	1.97	None	None		1.5
17	12.0	F.	0.67	—	1.58	—	None	0.608	2.24	None	—		1.6
18	12.6	F.	0.56	2.06	0.86	—	None	0.465	1.74	None	None		7.3
19	14.6	F.	1.24	1.92	1.09	1.06	None	Trace	1.52	None	None		8.7
20	14.2	M.	0.31	2.10	1.47	1.14	None	0.258	2.11	None		None	5.1
21	10.0	F.	0.31	1.92	0.64	0.96	0.065	0.144	2.03	None	None		4.0
22	15.5	M.	0.36	1.50	0.51	1.42	Trace	None	1.88	Trace		None	5.2
23	14.7	F.	0.41	1.94	0.80	1.78	Trace	Trace	2.07	Trace	None		11.0
24	23.4	F.	0.63	1.19	1.21	1.08	None	None	2.05	Trace	None		4.5
Average...			0.66	1.86	1.12	1.45	0.000	0.184	2.01	0.000	0.000	0.000	5.5

becoming mouldy or sour. A large "batch" could thus be made at one time.

Inasmuch as in these experiments our attention was directed mainly to the question of aluminium absorption and distribution in the tissues no attempt was made to determine exactly the quantities of [illegible]inium ingested. However, from calculation it is certain that for t[illegible]ight alum

TABLE 3

Aluminium content of the blood of dogs after a single meal of aluminium-containing food

25 to 31 inclusive and 38 and 39 = straight alum biscuit.
others = alum-phosphate biscuit. (Al as mgm. per 100 cc. of blood.)

TIME	DOG 25	DOG 26	DOG 27	DOG 28	DOG 29	DOG 30	DOG 31	DOG 32	DOG 33	DOG 34	DOG 35	DOG 36	DOG 37	DOG 38	DOG 39	DOG 40	DOG 41	DOG 42	DOG 43
2 days previous		0.82		Fasting			Ashed in porcelain	Fasting							Fasting				
1 day previous				1.10	1.06	Fasting				0.30	0.29			Fasting	0.62	Fasting, trace	0.000	0.000	0.058
Same day					1.04			0.88	0.77			0.38	0.58	0.77					
Fed																			
½ hour	0.55	0.92	0.54	1.87	1.06	1.48		0.36	0.78	0.36	0.22	0.39	0.56	0.55	0.73				
1 hour	0.38	1.04	0.55					0.78	0.78			0.52		0.51	0.54			0.000	0.099
2 hours	0.32									0.36	0.70		0.40			0.051	0.000		
3 hours		0.78	0.74					0.56	0.56			0.37		0.73	0.54			0.000	0.057
4 hours	0.86			1.19	0.43	0.92				0.28	0.53		0.65			0.048	0.000		
5 hours		0.96	0.72					0.74	0.84			0.72		0.54	0.53			0.000	0.029
6 hours	0.71				0.41	1.90				0.29	0.53		0.39			Trace	0.000		
7 hours			0.56					0.67	0.51			0.72		0.54	0.53			0.000	0.057
8 hours	1.06			1.04	0.40	0.97				0.74	0.22		0.39			Trace	0.000		
9 hours		1.17	1.24					0.72	0.91			0.22		0.73	0.45			0.000	0.043
10 hours				0.95	0.36	0.76				0.28	0.22		0.38			Trace	0.000		
24 hours		1.02	0.54	0.89	0.73	0.37		0.61	0.73	0.22	0.15	0.38	0.38	0.37	0.46	Trace	0.000	0.000	Trace
Average	0.64	0.95	0.67	1.16	0.68	1.06		0.66	0.73	0.35	0.35	0.46	0.46	0.59	0.55		0.000	0.000	0.049

biscuits the quantity of aluminium taken in was never less than 100 mgm. daily, and in most instances was much greater. The figures for the alum phosphate biscuits are probably somewhat lower than this.

Blood was drawn at specified intervals from the jugular vein into a Luer syringe containing aluminium-free oxalate. Enough was taken for an ash sample (usually about 10–20 cc.). When the animals were killed they were bled as completely as possible under ether anesthesia. The organs were removed carefully to avoid accidental contamination. The organs reserved were kidneys, liver, gall bladder, spleen, adrenals, ovaries, testes, brain, muscle and heart.

TABLE 3A

Aluminium content of tissues of dogs after a single meal of aluminium-containing food

Al as mgm. per 100 grams of wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
25	19.0	M.	0.84	1.99	—	1.34	None	0.487	1.23	0.48		—	2.6
26	15.0	M.	0.93	1.64	1.66	2.16	None	0.115	1.35	None		None	4.2
27	16.4	M.	0.50	1.91	0.53	2.07	0.032	0.136	1.19	—		None	2.8
28	16.8	F.	0.44	1.98	0.58	1.61	0.124	0.500	2.07	None	None		5.3
29	19.8	M.	—	1.75	1.21	1.18	0.065	0.132	2.07	None		None	5.2
30	11.8	F.	0.64	6.13	0.52	2.04	Trace	0.278	2.00	None	None		16.5
31	7.0	M.	0.87	2.49	0.98	2.00	0.114	0.283	1.99	None		None	3.4
32	15.6	F.	0.49	2.24	0.85	2.27	Trace	None	1.66	None	None		6.9
33	13.2	F.	0.84	2.63	0.48	1.56	None	0.280	2.52	None	None		3.2
34	14.0	M.	0.32	2.55	0.41	1.34	Trace	0.377	2.05	None		None	5.3
35	14.0	M.	0.61	2.29	0.62	1.78	Trace	0.570	2.65	None		None	—
36	22.0	F.	0.65	2.32	0.55	1.84	Trace	0.258	2.68	None	Trace		6.2
37	17.9	F.	0.49	3.38	0.59	1.20	None	0.365	2.66	None			5.2
38	20.0	M.	0.42	2.48	1.11	1.11	0.193	None	2.77	None		0.147	4.1
39	21.7	M.	0.42	1.68	0.67	1.14	0.125	0.206	2.82	None		0.167	4.4
Average			0.60	2.50	0.76	1.64	0.040	0.265	2.16	0.034	0.000	0.039	5.3

Blood samples were measured and dried immediately after drawing. Every care was taken to avoid accidental contamination of material. Analyses were made according to the method described in the preceding paper (10).

EXPERIMENTS. *Group I—Aluminium content of blood and tissues of fasting dogs.* There were twelve dogs in this series. They were starved for seven days. Distilled water was allowed freely. Blood samples were taken on the morning of the first, third, fifth and seventh day of starvation. After the last sample had been taken the dogs were killed according to the

TABLE 4

The aluminium content of the blood of dogs fed for one week on aluminium-containing food

Al as mgm. per 100 cc. of blood

DAY	HOUR AFTER FEEDING	STRAIGHT ALUM BISCUIT			ALUM-PHOSPHATE BISCUIT			CONTROL—YEAST BREAD	
		Dog 44	Dog 45	Dog 46	Dog 47	Dog 48	Dog 49	Dog 50	Dog 51
1	1	0.81			0.49			0.44	
	2		0.37			0.73			0.49
	3			0.39			0.36		
2	4	0.35			0.43			0.37	
	5		0.37			0.35			0.52
	6			0.38			0.38		
3	7	0.73			0.60			0.51	
	8		0.44			0.35			0.52
	9			0.38			0.45		
4	10	0.75			0.70			0.43	
	11		0.38			0.55			0.51
	12			0.39			0.52		
5	13	0.37			0.38			0.45	
	14		0.38			0.36			0.51
	15			0.39			0.52		
6	16	0.84			0.71			0.46	
	17		0.57			0.98			0.51
	18			0.37			0.57		
7	19	1.09			1.04			0.66	
	20								0.54
	21			1.47					
	Killed								
	HOURS AFTER LAST FEEDING								
	32	0.32	0.29	0.39					
	33	0.37				0.29			
	34				0.52		0.38		
	35							0.33	0.28
Average		0.63	0.39	0.52	0.60	0.51	0.45	0.44	0.46

procedure already described. The blood samples from the first six were ashed in porcelain and found to give doubtful results. These were discarded. The samples from the other six were ashed in silica. The organs of all were analysed as usual; the results for these are given here. All determinations were done in duplicate. In tables 1 and 1A are detailed the results for this group.

Group II—Aluminium of blood and tissues of dogs on a normal diet. Twelve dogs were kept without food for two days, then fed daily on a diet which was considered normal with respect to possible aluminium content. It consisted of hashed beef, yeast bread, and butter, and is herein

TABLE 4A

Aluminium content of tissues of dogs fed on aluminium-containing food for one week

Al as mgm. per 100 grams of wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
44	13.2	F.	0.64	2.22	0.44	1.34	0.026	1.62	1.95	1.02	None		9.2
45	17.2	M.	0.82	2.34	0.44	2.01	Trace	0.195	1.99	0.78		0.000	5.3
46	12.4	M.	0.68	2.31	0.53	1.82	0.111	0.188	2.23	None		0.000	2.9
47	10.3	F.	1.43	2.05	0.57	1.58	Trace	0.344	1.57	None	None		5.7
48	11.0	F.	0.98	1.78	0.55	1.25	None	0.353	1.28	None	None		9.3
49	10.6	M.	0.51	2.96	0.43	1.76	0.121	0.300	2.12	None		0.138	4.2
Average			0.84	2.27	0.50	1.63	0.043	0.500	1.85	0.30	None	0.046	6.1
50	9.2	F.	0.47	1.84	0.70	1.32	None	0.293	2.39	None	None		12.8
51	10.0	F.	0.42	2.20	0.80	1.31	Trace	Trace	2.51	None	None		8.1
Average (control)			0.44	2.02	0.75	1.31	0.000	0.147	2.45	0.000	0.000		10.4

designated the "control diet." The first six were bled only on the last day of the feeding period. The bloods were analysed by the trichloracetic acid filtrate method which was found to give unreliable results, hence are not included. The other six were bled on the first, fifth and eighth days, at various times after feeding, and were killed on the eighth day. All tissues were ashed in silica. The results may be seen in tables 2 and 2A.

Group III—Aluminium content of blood of dogs after a single meal of aluminium-containing food. This series consisted of nineteen dogs. They were fasted for one or two days, then given a single feeding of alum-phosphate or straight alum biscuit mixed with meat. Blood [illegible]s drawn at definite intervals after feeding. Blood samples of dog 31[illegible] ashed in

TABLE 3

Influence of four weeks' feeding of aluminium-containing food on the aluminium content of the blood

Al in mgm. per 100 cc. of blood

WEEK	HOUR AFTER FEEDING	STRAIGHT ALUM BISCUIT			ALUM-PHOSPHATE BISCUIT			CONTROL—YEAST BREAD	
		Dog 52	Dog 53	Dog 54	Dog 55	Dog 56	Dog 57	Dog 58	Dog 59
1	2	0.53			0.61			0.81	
	4		0.78			0.55			0.20
	6			0.93			0.50		
2	2	0.20			0.53			0.35	
	4		0.53						0.20
	6			0.63			0.32		
3	2	0.23			0.55			0.27	
	4		0.33			0.26			0.27
	6			0.47			0.27		
4	2	0.27			0.60			0.33	
	4		0.26			0.69			0.16
	6			0.28			0.28		
	Killed								
	HOURS AFTER LAST FEEDING								
	7	0.33	0.46		0.56	0.86			
	9			0.46			0.28		
	39							0.22	0.16
Average.....		0.31	0.47	0.55	0.57	0.59	0.33	0.40	0.20

Weekly averages—blood

WEEK	STRAIGHT ALUM	ALUM-PHOSPHATE	CONTROL
1	0.75	0.55	0.51
2	0.46	0.42	0.28
3	0.34	0.36	0.27
4	0.27	0.52	0.25
At death	0.41	0.56	0.19
Grand average...	0.45	0.48	0.30
	0.47		

porcelain, the results of which were discarded. The animals were killed after twenty-four hours. Tissues of fifteen of them have been analysed. The results are shown in tables 3 and 3A.

Group IV—Aluminium content of blood and tissues of dogs maintained upon aluminium-containing food for a period of one week. Eight dogs were fed for one week on a diet containing straight alum or alum-phosphate biscuit or yeast bread. Blood samples were taken each day at definite intervals after feeding. Since there was some indication in group III that greater absorption occurred in the later hours after feeding, it was planned to follow the course of absorption through the whole twenty-four hour cycle. Thus information might be gained concerning the time factor as

TABLE 3A

Aluminium content of tissues of dogs fed on aluminium-containing food for one month

Al as mgm. per 100 grams wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
52	11.0	F.	0.49	2.14	0.98	1.58	0.188	0.063	2.04	None	None		6.4
53	11.1	F.	0.52	2.07	0.76	0.97	0.117	None	1.35	None	None		3.1
54	8.6	M.	0.74	1.68	0.51	0.80	0.112	0.104	2.11	None		0.399	10.2
55	11.6	F.	0.50	1.42	0.71	1.48	None	0.508	1.82	None	None		6.0
56	13.0	M.	0.88	1.52	0.42	1.20	None	0.532	2.67	None		0.348	2.9
57	8.0	M.	1.35	1.70	0.48	1.17	0.153	0.528	2.59	None		0.263	2.5
Average.....			0.73	1.75	0.65	1.20	0.095	0.289	2.12	None	None	0.336	5.2
58	13.0	F.	0.66	1.32	0.79	1.20	0.028	0.139	1.65	None	None		4.6
59	16.0	F.	0.68	1.48	0.51	1.11	Trace	0.217	1.98	None	None		3.4
Average (control)..			0.67	1.40	0.65	1.15	0.014	0.178	1.82	None	None		4.0

well as the height to which the aluminium in the blood might possibly rise. For results see tables 4 and 4A.

Group V—Aluminium content of blood and tissues of dogs maintained upon aluminium-containing food for a period of four weeks. Eight dogs were fed for four weeks upon a diet identical with that of group IV. Blood samples were taken on the seventh day of each week at a definite time after the meal. Tables 5 and 5A show the results obtained.

Group VI—Aluminium content of blood and tissues of dogs maintained upon aluminium-containing food for a period of twelve weeks. Eight dogs were fed for twelve weeks. Blood samples were drawn at definite intervals

TABLE 6

The aluminium content of blood of dogs fed aluminium-containing food for twelve weeks

Al as mgm. per 100 cc. of blood

WEEK	HOUR AFTER MEAL	STRAIGHT ALUM BISCUIT			ALUM PHOSPHATE BISCUIT			CONTROL—YEAST BREAD	
		Dog 60	Dog 61	Dog 62	Dog 63	Dog 64	Dog 65	Dog 66	Dog 67
1	2	0.65						0.36	
	4		0.27			0.32			0.17
	6								
2	6	0.22						0.22	
	8		0.38			0.40			0.41
	10			0.32					
3	10	0.40			0.65				
	12		0.39			0.39			0.28
	14			0.43					
4	2	0.55			0.54			0.26	
	4		0.39			0.33			0.59
	6			0.33			0.32		
5	6	0.38			0.39			0.28	
	8		0.32			0.27			0.40
	10						0.39		
6	10	0.41			0.55			0.28	
	12		0.33			0.27			0.39
	14			0.33			0.36		
7	2	0.57			0.60			0.16	
	4		0.56			0.27			0.40
	6			0.42			0.28		
8	6	0.62			0.51			0.22	
	8		0.38			0.22			0.33
	10			0.44			0.38		
9	10	0.39			0.62			0.21	
	12		0.32			0.46			0.38
	14			0.43			0.39		
10	2	0.16			0.55			0.21	
	4		0.38			0.21			0.36
	6			0.39			0.28		
11	6	0.29			0.27			0.18	
	8		0.31			0.33			0.38
	10			0.44			0.28		

TABLE 6—*Concluded*

WEEK	HOUR AFTER MEAL	STRAIGHT ALUM BISCUIT			ALUM-PHOSPHATE BISCUIT			CONTROL—YEAST BREAD	
		Dog 60	Dog 61	Dog 62	Dog 63	Dog 64	Dog 65	Dog 66	Dog 67
12	10	0.27			0.27			0.22	
	12		0.31			0.21			0.25
	14			0.33			0.27		
Killed	25								
13	2				0.27				
	4								
	6			0.41			0.28		0.16
Killed	7			0.41					0.17
14	6				0.27				
	8								
	10						0.27		
Killed	23				0.24		0.32		
Average.....		0.41	0.35	0.39	0.44	0.30	0.32	0.24	0.33
		0.38			0.35			0.28	

TABLE 6A

Aluminium content of tissues of dogs kept for twelve weeks on an aluminium-containing diet

Al as mgm. per 100 grams wet tissue

DOG NUMBER	WEIGHT	SEX	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
60	8.9	F.	0.73	1.98	0.59	1.09	0.058	0.210	2.51	0.85	None		7.9
61	9.0	F.	1.05	2.56	0.52	1.55	0.028	0.166	2.47	1.31	None		7.2
62	10.3	F.	0.75	1.38	0.50	1.04	Trace	0.489	2.64	Trace	None		5.8
63	11.1	M.	0.70	2.83	0.60	2.52	None	None	2.54	0.25		—	4.6
64	15.0	M.	0.77	1.22	0.55	1.09	None	0.207	2.13	None		—	2.9
65	12.6	M.	0.97	1.82	0.48	4.19	None	0.065	2.20	None		None	8.2
Average			0.83	1.95	0.54	1.91	0.014	0.189	2.41	0.40	None	None	6.1
66	15.6	F.	0.84	1.61	0.55	1.45	Trace	0.100	2.14	Trace	None		5.1
67	16.2	F.	0.63	1.26	0.61	1.11	0.010	0.034	2.08	0.33	None		2.6
Average (control).			0.74	1.44	0.58	1.28	0.005	0.067	2.11	0.16	None		3.8

after the meal of the seventh day. Food was given at a regular time each day. The general condition of the animals was observed frequently. On the whole they ate well and remained in good nutritive condition. Three had to be replaced by others after the beginning of the experiment, one because it would not eat, two because they were vicious. The results are recorded in tables 6 and 6A.

In table 7 may be found average results for all groups of animals thus far considered.

TABLE 7

Summary of averages of determination of all groups

Al as mgm. per 100 grams wet tissue

PROCEDURE	NUMBER	LIVER	BILE	KIDNEY	BRAIN	MUSCLE	HEART	SPLEEN	ADRENAL	OVARY	TESTICLE	THYROID
1 wk. starving	12	0.94	1.90	0.84	1.25	0.114	0.192	1.84	0.51	None	0.000	7.25
1 wk. meat and bread	12	0.66	1.86	1.12	1.45	Trace	0.184	2.01	None	None	0.090	5.50
Single feeding	15	0.60	2.50	0.76	1.64	0.040	0.265	2.16	0.034	None	0.039	5.30
1 wk. feeding	6	0.84	2.27	0.50	1.63	0.043	0.500	1.85	0.300	None	None	6.10
1 wk. control	2	0.44	2.02	0.75	1.31	Trace	0.147	2.45	None	None		10.40
1 mo. feeding	6	0.73	1.75	0.65	1.20	0.095	0.289	2.12	None	None	0.336	5.20
1 mo. control	2	0.67	1.40	0.65	1.15	0.014	0.178	1.82	None	None		4.00
3 mo. feeding	6	0.83	1.95	0.54	1.19	0.014	0.189	2.41	0.400	None	0.000	6.10
3 mo. control	2	0.74	1.44	0.58	1.28	0.005	0.067	2.11	0.016	None		3.80

Discussion of results. *The aluminium content of blood and tissues of fasting dogs.* So far as one may glean from the literature, reliable data are lacking relative to the presence of aluminium in the blood of fasting animals. It has been stated at times that aluminium is a constant constituent of the tissues of animals and man, but equally positive statements have denied this (vide, e.g., McCollum et al., 11). From our own work with fasting dogs it is apparent (see table 1) that the blood of dogs fasted for seven days contains appreciable quantities of aluminium. The extreme limits of the concentration were 0.16 to 0.35 mgm. of aluminium per 100 cc. of blood, the average being 0.23 mgm. The delicacy of the method is such that these quantities must be regarded as significant. They cannot be laid to errors inherent in the method since experience has shown that the limit of error is a figure lying in the third decimal place, approximately 0.001, so small it may be disregarded.

The quantity of aluminium in the blood does not tend to diminish appreciably during this period of fasting. This must indicate previous storage in the body. In order that the amount in the blood remain at a

fairly constant level as fasting progresses there must be deposited somewhere in the body much larger quantities.

From an examination of table 1A it is evident that the notable places of storage for aluminium are the liver, kidney, brain and spleen.

The *liver* contains, on an average 0.94 mgm. per 100 grams of tissue, more than four times as much aluminium per unit as blood. The metal is, possibly, in the starving condition, on its way to excretion. The aluminium is released slowly, perhaps because the body is not capable of excreting it rapidly, or because the body has some use for it. Just how long the starvation would have to be prolonged in order to render the blood aluminium-free has not been determined, but it would seem, from an examination of the figures, that it might take a number of weeks. At the beginning of starvation the quantity in the organs may perhaps be assumed to have been larger.

The *kidney* is also a locus of storage, containing slightly less than the liver, and nearly four times (average) as much as the blood. That the kidney is also a channel for excretion of aluminium is to be inferred from the definite amounts found there. On the other hand, various investigators have failed to find aluminium in the urine of animals and man fed with aluminium containing foods. It is probable, however, that with a method sufficiently delicate, small quantities would have been found. In a subsequent paper evidence of the urinary excretion of aluminium will be presented.

That the kidney cannot be the most important excretory path is clear from the amounts found in the *bile*. Here, comparatively large quantities, more than eight times that found in the blood, are present, showing that aluminium is excreted principally through the bile, and that the liver of the fasting animal must be the organ chiefly concerned with picking out and excreting this metal. It is apparent that much of the aluminium excreted in this way may be reabsorbed.

In this group the *spleen* contains by far the greatest store, per unit, of aluminium, more than eight times the amount found in the blood. The large amount here cannot be attributed to the blood contained in the organ, especially as the animals were bled to death and the spleen was invariably found at autopsy to be quite contracted and hard. However, as this organ is very small as compared with the liver, its actual importance as a storage place for aluminium must not be over emphasized. But the fact that per unit it holds nearly twice as much as liver is interesting, however obscure the reason.

The *brain* also holds a larger store of aluminium per 100 grams of tissue than the liver and more than five and one-half times per unit as blood.

The *heart* and *voluntary muscle* contain relatively small amounts of aluminium. But when we consider the large mass of muscular tissue, as

compared with the spleen, for instance, it is evident that the total aluminium stored in the muscle mass may reach an important figure.

The *adrenals* contain very little aluminium, the *ovary* none, the *testicle* only a trace, if any. The large amount in the *thyroid* is very striking. It must be pointed out that with a tissue as small as adrenal, ovary or thyroid the error is multiplied greatly in the final calculation. But this is not the most important factor since the average for the aluminium actually present in the thyroid glands was 0.078 mgm., a significant figure. In the adrenals often no aluminium is found but there is always an appreciable amount present in the thyroid.

From these observations one is forced to conclude that aluminium occurs in the blood and tissues of animals maintained in a fasting conditions for a period of seven days. The spleen, brain, liver and kidney hold relatively large amounts, while the bile is richest of all in aluminium.

In view of these facts one must, then, consider aluminium as a constant constituent of the adult animal body. The bile may be regarded as the chief path of excretion of aluminium.

The aluminium content of the blood and tissues of dogs maintained on an ordinary (control) diet. When one turns to an examination of the results for the second group of dogs (table 2), those on a diet considered "normal," in that no aluminium had been added to it, one finds a slightly different state of affairs. The aluminium in the blood is, on the average, 0.13 mgm. per 100 cc. higher than in the fasting dogs, while the liver averages are lower by 0.28 mgm., the bile by 0.04 mgm., the kidney higher by 0.28 mgm., the brain by 0.20 mgm. and the spleen by 0.17 mgm. These differences, except for the bile, are significant.

The slightly higher value for the *blood* points either to absorption from food, or, in view of the smaller quantity found in the liver (table 2A), to an augmented release of the metal from the liver into the blood stream. The increase in aluminium may well come from the food ingested, since it has been shown that practically all of food substances may contain aluminium (12). Flour is particularly rich in this element. Whether the aluminium in foods is due to its natural occurrence or whether it may be regarded as a contamination cannot be decided at present. It is probable that with some foods both alternatives are correct.

In spite of the increase in the blood there is not a corresponding increase in the *bile*. The bile contains practically the same amount of aluminium as that of the fasting dogs. The ratio, however, between the content of the bile and liver is higher than for the fasting dogs, pointing, perhaps, to a more rapid excretion.

The quantity of aluminium found in the *kidney* is greater than that found in the kidney of the fasting dogs, and about twice as much as that found in the livers of the same group (II). This may mean increased

storage. Or it may merely be the result of the exposure of the kidney tissue to higher concentrations of aluminium in the blood. Not only that, but in view of the nutritional condition of the two groups of dogs, one might expect differences in the handling of any particular substance, so that in this case, the kidney may actually be more active in excreting the aluminium than it is in the starving animals.

That the *livers* of well fed animals may contain less aluminium than livers of starving ones is demonstrated. The explanation undoubtedly lies in the differences of the state of nutrition in the two groups. It may be possible that in the fasting animal changes in the composition of the liver have occurred which cause such a liver to yield an apparently higher content of aluminium than the liver of well fed animals, a liver which has not undergone a similar alteration in composition. It is interesting to note that the moisture content of the livers of the two groups varied somewhat. For starving animals 1 gram of dried liver was equivalent to 3.28 grams of wet tissue, for the fed animals the ratio was 1:3.46, (averages) showing a difference in composition with regard to the water found there. We know that the liver, during starvation, loses large stores of fat and glycogen. Each cell is a system maintaining chemical equilibrium with its surroundings. The loss of glycogen and fat must result in a more unstable equilibrium which tends to be adjusted in some way. All the ions which can be held are probably held tenaciously. Before a correct interpretation of these results can be made, therefore, a study of the influence of fasting on liver composition should be made.

The average figures for the *brain* and *spleen* in this group are higher than for the starving group, seemingly indicating that the tissue acts as a storage place for aluminium, which is taken from the blood by these organs.

The differences in the other organs are not striking. Only a trace of aluminium is found in the *muscle*, none in the *adrenal* or *ovary*. The difference in the thyroid we cannot explain.

The conclusions which may be drawn from this series of experiments are these: that the aluminium content of the blood of animals which have been fed on a diet to which no aluminium has been added, is slightly higher than for starving animals. The tissues examined also contained aluminium. The ratios between the values for aluminium are different, and point to a difference in the handling of this metal in the two nutritional states. As in starving animals the bile is a most important factor in the elimination of aluminium.

An "ordinary diet" in the sense that the term is used here, may be a source for the aluminium found in the blood.

The aluminium content of blood and tissues of dogs after a single feeding of food containing added aluminium. This section of the investigation was

an attempt to determine when aluminium first appears in the blood after a meal containing considerable amounts of it, and the period of greatest absorption. By this means it was hoped to establish definite laws for aluminium absorption. When the results are examined, however (table 3), it is found that variations in these factors are so great that no definite laws for aluminium absorption may be formulated. It was also hoped to determine any difference between the body's treatment of aluminium from alum-phosphate and straight-alum biscuits.

An examination of the results obtained from this group of animals brings to light interesting information relative to the behavior of aluminium in the body. The average aluminium content of the blood is higher than in either of the two preceding groups. Considering only averages, there is evidence that aluminium is absorbed more readily from food very rich in it than from an ordinary diet.

When one examines the data critically, however, wide variations from the average are seen to occur. This has not been observed in the two preceding groups. Also, many times the pre-feeding level is higher than in the control groups. If one uses the pre-feeding or the one-half hour level for comparison it is seen that the aluminium in the blood increases markedly in dogs 25, 27, and 30, moderately in dogs 26, 33, 34, 35, 36 and 43, and actually decreases in dogs 29, 32, 38 and 39, and does not appear at all in dogs 41 and 42.

When there is an increase the interval before its appearance differs greatly. In dog 25, after an initial decrease there was a rise in the fourth hour, the maximum occurring in the eighth hour. In dog 27 there was a gradual, slight rise then a fall, followed by a large increase in the ninth hour. In dog 30 the highest level was found in the sixth hour, in dog 35 at the end of two hours.

It is evident that wide variations in the aluminium content of the blood may occur at different times following the ingestion of food containing comparatively large quantities of it. The celerity with which aluminium appears in the blood after such a meal depends upon many factors, especially on the condition of the alimentary tract. If digestion were sluggish absorption might be retarded. If the aluminium-containing food, mixed with fairly large quantities of gastric juice, passed quickly into the intestines absorption might be very rapid.

That individual animals vary widely in their response to any given treatment is well known and must always be borne in mind. Dog 35 is perhaps the most interesting individual of this group. Not a robust or active animal, it ate less than any of the others, yet there was a prompt increase in the aluminium of the blood. This shows that the amount absorbed is not dependent on the absolute quantity of aluminium contained in the food.

For all these animals the rise of aluminium in the blood is very transitory, any such increase tending to disappear rapidly.

What may occur between the bleeding periods, or between the tenth and the twenty-fourth hour when the animals were killed, is not apparent. It is possible that those dogs showing little or no absorption might have exhibited an increase in the later hours. Gies (13) remarks that "the amount of aluminium in the blood at any moment after the ingestion of aluminized food, is but a fraction of the quantity that may occur in the blood of the individual during a given day—larger quantities circulate throughout the body meanwhile, under such conditions."

The fact that the blood of the animals showing little absorption (dogs 26, 28, 32, 38, 39) is generally much richer in aluminium than the others, suggests that previous to the experiment their diets had contained considerable quantities of aluminium. This fact may also be interpreted to mean that the blood or indeed any of the tissues may become, as it were, saturated with a given metallic ion for the time being and be then unable to carry any further added increment. Such a condition has been suggested for arsenic and there is no apparent reason why this explanation would not hold for aluminium.

The response of the animals to straight alum biscuit does not differ materially from the response to alum-phosphate biscuit. While the average for blood is slightly higher it can hardly be regarded as evidence of greater absorption since the values both at the beginning and end are consistently higher.

The *livers* (see table 3A) in this group show definitely less aluminium than the fasting, and slightly less than the control group. This, when the higher figure in the *bile* is considered, points to increased activity of the liver in picking out and excreting large quantities. This explains, in part, why the aluminium does not attain an even higher concentration in the blood, and why a high concentration is not maintained long.

That the *kidney* contains less aluminium than do the kidneys of the other group suggests that the body employs the most efficient means for the excretion of large amounts of this metal.

The quantity of bile excreted by dogs has been determined (14). It varies from 2.9 to 36.4 grams per kilogram of body weight. For the average weight of this group, 16.2 K., that would be 36.9 to 589.7 grams. If we assume, for the sake of illustration, that the bile as it is excreted, contains as much aluminium as that found in the gall bladder twenty-four hour after feeding, it would mean an actual excretion of 0.93 to 14.6 mgm. of the metal for twenty-four hours (using the average figure for bile). While these figures are only approximate, they serve to illustrate the point that surprisingly large quantities of aluminium may be excreted in this way.

The average aluminium content of *brain* and *spleen* is slightly higher than for the previous groups, as one might expect, realizing that the tissues have been exposed to a blood richer in it. The changes in the other tissues are less remarkable.

From these experiments it is evident that aluminium is absorbed from food to which it has been added, but the quantity appearing in the blood does not reach a high level. The rise is transitory, the quantity tending to diminish rapidly. The aluminium residues in either type of biscuit, supposed to be aluminium phosphate in one case and aluminium hydroxide in the other, must be in part soluble, and the aluminium from either source is absorbed.

Aluminium is found in the most important organs of the body, in the brain and spleen of these animals in larger quantities than in controls, in the liver and kidney less. The bile is, however, richest in aluminium and must be considered a most important excretory path.

Aluminium content of blood and tissues of dogs maintained for one week on a diet to which aluminium had been added. Table 4 shows what was suggested before, that absorption tends to be greater in the later hours after the ingestion of aluminized foods. In dogs 44, 46 and 47 there are marked increases in the aluminium of the blood in the later hours of the last two days. In dogs 45, 48 and 49 the aluminium rises slightly. Even dog 50, a "control," shows the same tendency. While the averages here are not much higher than for the normal animals of this series or of group II, this relatively large increase in the sixth and seventh day must be taken to mean an increase in absorption in the later hours after feeding. That the level tends to drop quickly is shown again by the low figures at death. It is also seen that the organism did not distinguish between the two forms of aluminium administered. Both were absorbed. More is absorbed when the quantity in the food is large.

The aluminium found in the *liver* (see table 4A) is higher than for any previous group of animals excepting the fasting dogs, and the kidney is lowest for all groups. The amount in the *bile* is large, although not as large as in the preceding group. Again the *brain* and *spleen* hold appreciable amounts, but the difference between the values and those for the previous groups is not striking. Compared with control animals of the same group (dogs 50 and 51), this group shows more aluminium in the liver, bile and brain, and less in the kidney and spleen than the controls. Why the value for spleen in these two control dogs is so much higher than for any other group we cannot explain. Nor can we explain the very large aluminium content of the thyroid gland of these two dogs. There are no very remarkable differences in the other organs.

The quantity of aluminium in the *bile* is less than in the single feeding group, but this seems to be balanced by the increase in the liver. There

is less aluminium in the kidneys of this group than in any other, indicating that in this case at least the kidney is not so greatly concerned with the elimination of aluminium as the bile and liver.

It is interesting to note that the control animals maintained the same order of aluminium storage as in group II and group III, that is, liver, kidney and bile, while the experimental animals have established a new order of magnitude for storage,—kidney, liver and bile. This indicates again the use of the most efficient means of removing large quantities of aluminium. It must be mentioned again that when the animal body is subjected to large excesses of aluminium for a prolonged period, any one organ may quickly become saturated with this metal, so that even large quantities may exist in the blood, but the quantity in any particular organ may not increase.

This series of experiments leads us to the same conclusions we have reached before, that aluminium is absorbed from foods containing it, and appears in the blood and certain tissues, that the liver and bile are the organs chiefly concerned with its excretion. In addition we may conclude that the greatest absorption occurs after the fifteenth hour following the ingestion of food containing large quantities of aluminium.

The aluminium content of blood and tissues of dogs maintained on an aluminium-rich diet for four weeks. By prolonging the period of feeding of excessive quantities of aluminium one should be able to show whether the animal organism will establish a "tolerance" for the metal, whether some alteration in the method of dealing with it will occur, or whether the aluminium content of the blood and tissues can be pushed to still higher levels.

Table 5 shows the results obtained by the feeding of large quantities of aluminium for a period of one month. Here the changes are very striking. Considering first the average figures for the blood of dogs 52, 53 and 54, it is seen that the aluminium found in the blood the second, fourth and sixth hours after feeding diminishes steadily, week by week, so that by the fourth week there is found little more than one-third the amount found in the first week in the same time interval. The individuals showing this very strikingly are dogs 53 and 54. A similar thing happens in the case of the alum-phosphate animals (55, 56 and 57) except that the level tends to rise slightly after the third week. Dog 56 illustrates this especially well. On the whole, however, the tendency is distinctly toward a lowering of the quantity of aluminium in the blood.

This points to a decreased absorption. But absorption is still probably taking place as some of the figures obtained at the death of the animal show. It would seem that the tendency is for less and less aluminium to be absorbed as the weeks go on, but the process does not stop, it merely takes place more slowly. Possibly the permeability of the capillaries of

the intestinal wall has been altered by exposure to excessive quantities of aluminium compounds over a long period.

While there is some variation among individuals of the same group, these variations are not very striking, and the averages obtained are lower than for the dogs of groups 3 and 4.

The aluminium content of the blood of the two control dogs decreases also, but this can hardly be considered evidence of lowered aluminium absorption, but rather that, with a food relatively free of aluminium, such amounts of the metal as may have been stored in the body have tended to be eliminated. The aluminium in the blood of dogs 58 and 59 fell to the level for fasting dogs.

A decrease in the absorption of aluminium is evidenced by the slight decrease in the aluminium of the liver (see table 5A) and the marked decrease in the bile as compared with dogs of groups III and IV. Dogs 52, 53, 55 and 58 show more aluminium in the kidney than in the liver, while the others show more in the liver.

Considering all six aluminium fed animals together it is evident that although all the values are higher, with the exception of the kidney, than the control animals of this series the differences are less marked than in the other two groups (III and IV).

One might expect to find largely augmented quantities in some other organ to account for the smaller quantities found here. It has been said that the spleen and pancreas are especially rich in aluminium after the prolonged administration of food containing it (15). Apparently this is not correct for the spleen has little more than that of the control animals of this group, and about the same amount as those obtained from dogs fed aluminized food only once. Nor has the brain content increased, but has rather tended to decrease, when compared with that for the single feeding or one week dogs. The changes in the other organs do not seem remarkable.

In general, the evidence seems to point to a decreased absorption when a diet containing large amounts of aluminium is fed over a protracted period. The lessened quantity in the bile possibly indicates lessened excretion because of a lower blood level.

Aluminium content of blood and tissues of dogs maintained on an aluminium-rich diet for twelve weeks. This experiment was planned with the same factors in mind as in the preceding group. It was desired to determine by what mechanism the organism adapts itself to the long continued administration of aluminium.

Table 6 shows the results obtained for the blood after a twelve weeks period of such feeding. In general the results coincide with those for the preceding group. The aluminium in the blood tends to decrease as the weeks pass. There is some variation which is not dependent on the time when the blood samples were taken, or on the condition of the animals.

This experiment was done during the summer months. Usually the animals ate well the first three or four weeks, then the appetite failed, they might even refuse to eat for two or three days. Usually when appetite failed markedly milk was given for a few days in addition to the food. This generally resulted in improvement in appetite. Sometimes yeast was also given.

Dog 64, showing the lowest average for the whole period began to lose appetite very soon after the experiment began. A skin disease developed, the animal being covered with abscesses which became large bleeding sores. It became so sick it would not attempt to eat or even move. It was given milk, a quart at a time, by stomach tube, for a few days. Improvement started at once, by the end of the experiment it was eating all its food, the skin had healed and new hair was growing. Even under these various conditions the aluminium in the blood did not change much, maintaining a fairly constant low level after the third week except for the ninth week when it rose 100 per cent, then dropped again.

Dogs 63 and 65 maintained their appetites and good nutritional condition during the entire period of the experiment, appetite beginning to fail only in the last week.

That the quantity of aluminium in the blood is not dependent on the absolute amount ingested is again illustrated. Dog 60 ate well the first four weeks, the blood value varying during the time from a high figure to a low one. The seventh week, when the animal was not eating well, a high figure appears. In the later weeks, after the appetite had improved, the aluminium falls to a fairly low level.

By comparing the average blood content of this group with that of group V, it is found that the values are practically the same except for the alum phosphate dogs, for which the level is slightly lower. The averages for blood of dogs fed both types of biscuits are low in this experiment, but still slightly higher than for controls, indicating that even though absorption has decreased markedly it has not stopped altogether.

As in the preceding groups (see table 6A), liver and bile are chiefly concerned with excretion and storage. The average content of all the organs tends to be slightly higher for the twelve week animals than for the four week ones, except for controls, which are much the same in the two groups. While absorption has seemed to be decreased, storage in the organs has gone on, as well as efficient excretion in the bile.

The experiments of Myers and Morrison (16) in so far as they are comparable with our own show quantitative rather than qualitative differences.

General discussion. These experiments contain many interesting suggestions relative to the behavior of the animal body toward aluminium, and perhaps toward metallic ions in general.

We know the absurdity of crediting to the absorbing elements of the gastro-intestinal tract any occult discretionary power with respect to the

substances to which they are exposed. The body absorbs substances, regardless of their beneficient or harmful effects, in accordance with physico-chemical laws. The process goes on in a definite way, and depends on the relative concentrations and solubilities in the various components of the body. The tendency with respect to any given substance is for a shifting equilibrium to be established between the various organs, the blood, the lymph and the gastro-intestinal tract.

In these experiments such an equilibrium is definitely indicated. It is seen that the aluminium content of the blood shifts from higher to lower levels during the experiments, depending in a general way on the amount of aluminium ingested, with the exception of the last two experiments, when another factor, namely, permeability of the absorbing elements, has presumably altered.

It is apparent also that the equilibrium is changed with some difficulty since the ingestion of large amounts of aluminium-containing foods does not often result in a large increase in the blood or tissues. The general trend is toward an increase, but the establishment and maintenance of a high blood level by means of feeding seems impossible.

While aluminium was found in the blood of all but two of the animals tested we are unwilling to state that aluminium is a "physiological" metal in the sense that calcium or magnesium is. Since the amount varies considerably we are inclined toward the viewpoint that it should be classed rather with copper and arsenic.

If it should be proved a "physiological" metal, that is, a necessary and indispensable ion, it is difficult to define just what the function might be. Stoklasa (17) assigns to aluminium important functions in plant life, especially in photosynthesis, germination and growth. Balls (18) recalls the suggestion of Papillon that aluminium may act as a substitute for metals, notably iron, in which the diet is deficient. Balls himself, on the basis of rather scanty evidence, suggests that aluminium may replace iron in ferruginous proteins. Bertrand (19) names aluminium as one of the metals always occurring in the animal body, which may act as catalysers in vital processes. That it does not catalyse reactions occurring during muscular activity is suggested by its occasional absence from both heart and voluntary muscle. There have been suggestions that aluminium plays a rôle in reproduction (20) (21).

Table 7 summarizes the averages for the various groups. While caution is necessary in drawing conclusions from averages, this method is more fair, considering the large number of animals, than comparing individuals. It is to be expected that individuals may occasionally be found which show the contrary trend. In this discussion we confine ourselves entirely to these larger, more obvious and general tendencies.

Considering the organs first in respect to the aluminium *per unit* of *tissue* we are surprised at the very high result for the thyroid, which

contains nearly three times as much as the next highest tissue. But the thyroid, as has been pointed out before, because of its very small size, cannot be considered an important storage depot for aluminium. But the fact that this tissue picks out aluminium in such large amounts would seem to have some significance. Is aluminium necessary to the metabolism of the thyroid? Is aluminium a vital constituent of the gland or a fortuitous impurity attached to colloids? That this is not a property of glands in general is shown by the relatively small amount in the other glands examined, namely, adrenal, ovary and testicle. No aluminium was found in any of the ovaries examined. It is sometimes present, sometimes absent in the testicle and adrenal. In the adrenal the amount present has no discernible relation to the experimental procedure.

Still considered on the basis of unit content, the spleen is the second richest organ, containing over 50 per cent more aluminium than the brain, which in turn contains nearly 90 per cent more than the liver and kidney.

The bile is, in every case, very rich in aluminium. This shows conclusively that here is the most important channel for excretion. It is probable that some of this excreted aluminium is again absorbed.

When the aluminium stores of the organs are considered in order of actual importance because of size, the liver must be given first place. The brain is next in importance with the kidney and spleen nearly equal, for while the spleen weighs less than the kidney its aluminium content is greater. The heart and voluntary muscle are much less important as storage depots for aluminium.

The general differences noticed between the animals fed once, or for one, four or twelve weeks merely illustrate different stages in the same process, namely the physiological adjustment to the continued administration of large amounts of aluminium in the food. At first, after a single feeding, the body adjusts by absorbing relatively large quantities, storing a large share in the brain, spleen and other tissues, and excreting much through the bile. At the end of one week's feeding the body is still adjusting in much the same way and to about the same extent. But after a month's exposure to this treatment absorption is definitely decreasing and storage and excretion concomitantly, though excretion through the bile is still large. After three months, absorption has decreased further, but the aluminium absorbed is treated in the same manner as before.

We are not in a position to state whether decreased absorption means decreased permeability, that is, injury, of the capillaries of the intestinal tract. This may very well be another purely normal physiological adjustment. On the other hand, can lessened absorption be regarded as a protective mechanism against possible injury by aluminium? Its behavior in this respect is strikingly like that of arsenic. There was no

evidence, from observation of our animals, of any impairment in health due to these dietaries.

CONCLUSIONS

1. Aluminium occurs in the blood and tissues of normal fasting dogs.

2. Aluminium occurs in slightly larger amounts in the blood and tissues of dogs fed a diet to which no aluminium had been added. Therefore aluminium is present in and absorbed from the ordinary diet.

3. Aluminium is promptly absorbed in small quantities following a single feeding of food to which it has been added.

4. Aluminium continues to be absorbed when aluminium-rich diets are fed for various periods of time.

5. After prolonged periods of feeding with food rich in aluminium, absorption of the metal decreases.

6. Concomitant with the decrease in absorption there is a decrease in storage and excretion.

7. The aluminium absorbed circulates in the blood, is stored especially in the liver, brain, kidney, spleen and thyroid.

8. The bile is the chief excretory path for aluminium.

BIBLIOGRAPHY

(1) Smith, E. E. Aluminium compounds in food. (Hoeber) 1928, New York.
(2) Myers, C. N. and C. Voegtlin. Pub. Health Reports, 1914, xxix, 1625.
(3) Kastyal, L. Magyar Orvasi Archivum, 1926, xxvii, 237. From C. A.
(4) Archangelski, V. M. Arch. Biol. Sciences, 1924, xxiv, 163.
(5) Abrahamson, E. M. and E. G. Miller, Jr. Proc. Soc. Exper. Biol. Med., 1925, xxii, 438.
(6) Britton, H. T. S. Journ. Chem. Soc., 1925, cxxvii, 2110.
(7) Blum, W. Journ. Amer. Chem. Soc., 1916, xxxviii, 1282.
(8) Greenfield, R. E. and A. M. Buswell. Ibid., 1922, xliv, 1435.
(9) Connor, S. D. Journ. Amer. Soc. Agron., 1921, xiii, 113.
(10) Underhill, F. P. and F. I. Peterman. This Journal, 1929, xc, 1.
(11) McCollum, E. V., O. S. Rask and F. E. Becker. Journ. Biol. Chem., 1928, lxxvii, 753.
(12) Langworthy, C. F. and P. T. Austin. The occurrence of aluminium in vegetable products, animal products and natural waters. New York, Wiley, 1904
(13) Gies, W. J. Journ. Amer. Med. Assoc., 1911, lvii, 816.
(14) Hammarsten, O. and S. G. Hedin. Physiological chemistry. Transl. by J. A. Mandel, 7th ed., Wiley, 1915, p. 414.
(15) Kahn, M. Biochem. Journ., 1912, ii, 235.
(16) Myers, V. C. and D. B. Morrison. Journ. Biol. Chem., 1928, lxxviii, 615.
(17) Stoklasa, J. Über die Verbreitung des Aluminiums in der Natur. Fischer, Jena, 1922.
(18) Bates, A. K. Dissertation. Columbia, 1920.
(19) Bertrand, G. Bull. Soc. Sci. Hyg. Alimentaire, 1920, viii, 49.
(20) Hoffman, A. L. and M. K. Hutton. Journ. Biol. Chem., 1925, lxiii, 143.
(21) Mitchell, H. S. and L. Schmidt. Journ. Biol. Chem., 1926, lxx, 471.

STUDIES IN THE METABOLISM OF ALUMINIUM

IV. The Fate of Intravenously Injected Aluminium

FRANK P. UNDERHILL, F. I. PETERMAN AND S. L. STEEL

From the Department of Pharmacology and Toxicology, Yale University, New Haven, Conn.

Received for publication April 19, 1928

The fate of aluminium after intravenous injection has not been extensively studied. Steel (1), in four dogs, found 5.27 to 11.11 per cent of intravenously injected aluminium in the feces in the three days following the injection. The remainder is not accounted for.

Recently Myers and Morrison (2) have shown that when aluminium is parenterally introduced into dogs it is widely distributed in the tissues and is only slowly excreted.

Aluminium is very gradually absorbed by the dog from aluminium-rich food, the quantity in the blood seldom rising to a high figure (3). Thus far one has been unable to cause a large and sudden increase in the blood value by means of feeding. In the present study an attempt has been made to learn in what ways the body would react to a sudden increase in the aluminium content of the blood. An amount of aluminium was injected which was slightly greater than the largest amount found in the blood after feeding aluminium-rich food. It was planned to follow the rate of disappearance from the blood and the time of appearance in other body fluids, as well as the rate of increase and decrease.

PROCEDURE. Dogs under morphine-atropine-ether, or amytal anesthesia were used. The femoral vein was exposed for injection purposes. It was not ligatured, injection being made slowly through a hypodermic needle from a burette. The femoral artery on the opposite side was exposed for blood collection. This was usually clamped off, but a free flow of a few cubic centimeters of blood was allowed before the sample was taken. Blood samples were collected directly into Ostwald pipettes, no oxalate being used.

Urine was collected by means of a catheter. Lymph was obtained from the thoracic duct. Bile was collected from the common bile duct after the gall bladder had been tied off.

Both lymph and bile were collected directly into weighed silica crucibles and samples weighed. When bile was being collected, 15 to 20 cc. of 0.4 per cent hydrochloric acid was injected into the duodenum to stimulate the flow. This was not usually repeated.

Stomach and intestinal loops were made by the insertion of large cannulae. The entire stomach and large intestine were washed, and a loop of small intestine about three feet in length, below the entrance of the bile duct, was washed with warm saline, the last washing before the injection being used as a control sample. In only one case did any of the washings contain more than a small trace of blood.

Every precaution was taken to prevent contamination of samples.

The solutions used for injection were aluminium sulphate or aluminium chloride dissolved in normal saline. In one case 10 mgm. of aluminium per kilo were given but the dog died suddenly, and the aluminium of the blood was too high to be accurately estimated by the method used. The usual amount given was 1 or 2 mgm. of aluminium per kilo. The injection was made slowly. The animal was kept warm.

ANALYTICAL METHODS. Aluminium was determined by means of the method already described (4).

Phosphate was determined by a modification of the Bell-Doisy (5) method as follows: 50 cc. of the saline washings were evaporated to dryness in a silica crucible and ashed at a low red heat. The ash was dissolved in 5 cc. of 2 per cent trichloracetic acid and 1 cc. of sodium sulphite, 2 cc. molybdate solution and 1 cc. of hydroquinone were added. Standards prepared at the same time contained 1 to 5 cc. of standard phosphate solution in a final volume of 9 cc. All phosphorus was thus determined as inorganic phosphate.

RESULTS. The protocols reproduced below illustrate the procedure and show the results obtained.

I. The examination of blood, urine and bile.

Experiment A

Dog, weight 14.2 kgm. Intravenous injection of $AlCl_3$ solution. Given 2 mgm. Al per kilogram = 71.0 cc. solution = 28.4 mgm. Al.

TIME	BLOOD, Al/100 cc.	BILE, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
12:20	0.18		Trace	Normal. 15 cc. 0.4 per cent HCl injected into duodenum
12:35		None		Normal
12:40				Injection begun
1:00		0.72		
1:08				Injection finished
1:10	4.32			All urine pressed from bladder
1:24		0.83		
1:38	4.16			½ hour
2:00		0.70		

Experiment A—concluded

TIME	BLOOD, Al/100 cc.	BILE, Al/100 GRAMS	URINE Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
2:08	4.01			1 hour
2:40			2.58	Volume 32 cc. Total excretion 0.83 mgm.
2:50		1.16		
3:08	3.54			2 hours
3:35		0.79		
4:18		0.70		
4:30			1.29	Volume 35 cc. Total excretion 0.45 mgm.
5:08	3.37	0.74		4 hours
5:55		0.66		
6:08	3.19			5 hours
6:55		0.62	1.57	Volume 48.5 cc. Total excretion 0.76 mgm.
7:08	2.69	1.06		6 hours

Total urine volume = 115.5 cc.
Total aluminium in urine = 2.04 mgm.
Total bile = 14.978 grams
Total aluminium in bile = 0.110 mgm.

Experiment B

Dog, weight 16.3 kgm. Intravenous injection $AlCl_3$ solution, 5 cc. = 2 mgm. Al. Given 1 mgm. Al per kilogram.

TIME	BLOOD, Al/100 cc.	BILE, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
9:45			None	Normal
1:35	0.21		None	Normal; 20 cc. 0.4 per cent HCl injected into duodenum
2:30		0.196		Normal
2:32				Injection begun
2:57				Injection finished
3:27	2.51			½ hour
3:57	2.38			1 hour
4:22			2.60	Volume 10.2 cc. Excretion 0.27 mgm.
4:27	2.64			1½ hours
4:57	2.68	0.372		2 hours
5:27	2.60			2½ hours
5:57	2.72			3 hours
6:30			1.95	Volume 11.9 cc. Excretion 0.21 mgm. Al
6:57	2.79			4 hours
7:57	2.82			5 hours; 5 mgm./kgm. morphine subcutaneously
8:57	2.03			6 hours
9:57	1.06	1.010		7 hours

Total urine excretion = 22.1 cc.
Total aluminium in urine = 0.48 mgm.
Aluminium in liver = 0.85 mgm. per 100 grams
Total bile = 7.054 grams

II. The examination of blood, urine and lymph. In the first three of the following experiments aluminium solutions were injected, in the last the aluminium was administered per os in baking powder biscuits. In the first experiment an excessive dose of aluminium chloride was given and the dog died suddenly before the full amount had been injected. The protocol is included however as illustrating the excretion of aluminium through the lymph.

Experiment C

Dog, weight 13.6 kgm. Intravenous injection of $AlCl_3$ solution, 5 cc. = 10 mgm. Al. Given 10 mgm. per kilogram.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
11:30		None		Normal
12:00	0.04	None	Trace	Normal
12:35				Injection begun; 1 cc. per minute
1:10	5.81	2.16	0.067	Died suddenly

Experiment D

Dog, weight 11.1 kgm. Intravenous injection of $AlCl_3$, 5 cc. = 2.9 mgm. Given 1 mgm. per kilogram. Amytal anesthesia.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
2:30	0.21	Trace	None	Normal
2:40				Injection begun
3:00				Injection finished
3:15		0.48		
3:30	1.87			½ hour
3:35		0.45		
4:00	1.82			1 hour
4:04		0.25		
4:30	1.82			1½ hours
4:44		0.33		
5:00	2.22			2 hours
5:15		0.28		
6:00	2.05			3 hours
6:15		0.21		
6:20			4.82	Volume 14.7 cc. Excretion 0.71 mgm. Al
7:00	2.05			4 hours
8:00	2.13	0.28		5 hours
9:00	2.06	0.39		6 hours
9:10			2.32	Volume 8.0 cc. Excretion 0.19 mgm.
9:30	1.87			6½ hours

Total urine excretion = 22.7 cc.
Total aluminium in urine = 0.89 mgm.
Total lymph = 56.68 grams
Total aluminium in lymph = 0.160 mgm.
Aluminium content of liver = 0.68 mgm. per 100 grams

TIME	BLOOD, Al/100 cc.	BILE, Al/100 GRAMS	URINE Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
2:08	4.01			1 hour
2:40			2.58	Volume 32 cc. Total excretion 0.83 mgm.
2:50		1.16		
3:08	3.54			2 hours
3:35		0.79		
4:18		0.70		
4:30			1.29	Volume 35 cc. Total excretion 0.45 mgm.
5:08	3.37	0.74		4 hours
5:55		0.66		
6:08	3.19			5 hours
6:55		0.62	1.57	Volume 48.5 cc. Total excretion 0.76 mgm.
7:08	2.69	1.06		6 hours

Total urine volume = 115.5 cc.
Total aluminium in urine = 2.04 mgm.
Total bile = 14.978 grams
Total aluminium in bile = 0.110 mgm.

Experiment B

Dog, weight 16.3 kgm. Intravenous injection $AlCl_3$ solution, 5 cc. = 2 mgm. Al. Given 1 mgm. Al per kilogram.

TIME	BLOOD, Al/100 cc.	BILE, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
9:45			None	Normal
1:35	0.21		None	Normal; 20 cc. 0.4 per cent HCl injected into duodenum
2:30		0.196		Normal
2:32				Injection begun
2:57				Injection finished
3:27	2.51			½ hour
3:57	2.38			1 hour
4:22			2.60	Volume 10.2 cc. Excretion 0.27 mgm.
4:27	2.64			1½ hours
4:57	2.68	0.372		2 hours
5:27	2.60			2½ hours
5:57	2.72			3 hours
6:30			1.95	Volume 11.9 cc. Excretion 0.21 mgm. Al
6:57	2.79			4 hours
7:57	2.82			5 hours; 5 mgm./kgm. morphine subcutaneously
8:57	2.03			6 hours
9:57	1.06	1.010		7 hours

Total urine excretion = 22.1 cc.
Total aluminium in urine = 0.48 mgm.
Aluminium in liver = 0.85 mgm. per 100 grams
Total bile = 7.054 grams
Total aluminium in bile = 0.31 mgm.

following experiments aluminium solutions were injected, in the first the aluminium was administered per os in baking powder biscuits. In the first experiment an excessive dose of aluminium chloride was given and the dog died suddenly before the full amount had been injected. The protocol is included however as illustrating the excretion of aluminium through the lymph.

Experiment C

Dog, weight 13.6 kgm. Intravenous injection of $AlCl_3$ solution, 5 cc. = 10 mgm. Al. Given 10 mgm. per kilogram.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
11:30		None		Normal
12:00	0.04	None	Trace	Normal
12:35				Injection begun; 1 cc. per minute
1:10	5.81	2.16	0.067	Died suddenly

Experiment D

Dog, weight 11.1 kgm. Intravenous injection of $AlCl_3$, 5 cc. = 2.0 mgm. Given 1 mgm. per kilogram. Amytal anesthesia.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
2:30	0.21	Trace	None	Normal
2:40				Injection begun
3:00				Injection finished
3:15		0.48		
3:30	1.87			½ hour
3:35		0.45		
4:00	1.82			1 hour
4:04		0.25		
4:30	1.82			1½ hours
4:44		0.33		
5:00	2.22			2 hours
5:15		0.28		
6:00	2.05			3 hours
6:15		0.21		
6:20			4.82	Volume 14.7 cc. Excretion 0.71 mgm. Al
7:00	2.05			4 hours
8:00	2.13	0.28		5 hours
9:00	2.06	0.39		6 hours
9:10			2.32	Volume 8.0 cc. Excretion 0.19 mgm.
9:30	1.87			6½ hours

Total urine excretion = 22.7 cc.
Total aluminium in urine = 0.89 mgm.
Total lymph = 56.68 grams
Total aluminium in lymph = 0.160 mgm.
Aluminium content of liver = 0.68 mgm. per 100 grams

Experiment E

Dog, weight 15.6 kgm. Intravenous injection of $AlCl_3$ solution, 5 cc. = 1 mgm. Al. Given 1 mgm. per kilogram.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	
3:15		None	No urine excreted	Normal
4:00	None	None		Normal
4:01				Injection begun
5:00		0.26		
5:05				Injection finished
5:30		0.35		
5:35	1.13			30 minutes
6:00		0.61		
6:05	1.15			1 hour
6:30		0.60		
7:05	1.11			2 hours
7:30		0.58		
8:05	1.11			3 hours

Aluminium content of liver = 0.58 mgm. per 100 grams
Total lymph = 46.458 grams
Total aluminium in lymph = 0.056 mgm.

Experiment F

Dog, weight 9.6 kgm. Fed meat and alum-phosphate biscuit. Amytal anesthesia.

TIME	BLOOD, Al/100 cc.	LYMPH, Al/100 GRAMS	REMARKS
	mgm.	*mgm.*	
6:50 a.m.			Fed
7:22			Amytal
10:25			Lymph collecting begun
10:55		0.114	
11:00	0.000		
11:25		0.087	
11:40	0.000		Lymph cannula cleaned
11:55			
12:30		0.079	
1:30	0.000	0.061	
2:30	0.000	0.060	
3:30	0.000	0.064	
4:30	0.000	0.101	

Total lymph = 45.78 grams
Total aluminium in lymph = 0.035 mgm.
Total urine excretion = 14.50 cc.

III. Excretion through the walls of the intestinal tract. The following experiments illustrate the excretion of aluminium through the walls of the intestinal tract. In the first experiment aluminium only was determined. In the others phosphate was also determined. In the last experiment the injection fluid consisted of normal saline only and thus constituted a control.

Experiment G

Dog, weight 10.0 kgm. Intravenous injection of $Al_2(SO_4)_3$, 10 cc. = 1.0 mgm. Al. Given 1 mgm. Al per kilogram.

TIME	STOMACH TOTAL Al	SMALL INTESTINE TOTAL Al	LARGE INTESTINE TOTAL Al	URINE, Al/100 cc.	REMARKS
	mgm.	*mgm.*	*mgm.*	*mgm.*	
9:30					Amytal
11:30					Ether
12:30	0.000	0.000	0.000	0.000	Completion of first washing of loops
12:32					Injection begun
12:58					Injection finished
6:15	0.017	0.050	0.067	2.050	Second washing

Notes: January 10—Dog not fed
January 11—Dog not fed
January 12—Dog given 15 grams $MgSO_4$
January 13—Experiment performed
Total urinary excretion 12:30–6:30 = 30 cc.

Experiment H

Dog, weight 11.1 kgm. Intravenous injection of $AlCl_3$ solution, 10 cc. = 1 mgm. Al. Given 1 mgm. Al per kilogram.

TIME	ALUMINIUM TOTAL			PHOSPHORUS TOTAL			REMARKS
	Stomach	Small intestine	Large intestine	Stomach	Small intestine	Large intestine	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Feb. 20							Dog not fed
Feb. 21							15 grams $MgSO_4$
Feb. 22							
7:15							Amytal
10:55	Trace	0.39	0.002	0.19	0.38	0.17	Normal control samples
10:58							Injection begun
11:28							Injection finished
2:10	Trace	1.28*	0.007	1.41	23.2*	1.56	Died. Intestinal tract congested

* Washings contained much blood.

Experiment I

Dog, weight 16.0 kgm. Intravenous injection of $Al(SO_4)_3$, 10 cc. = 1 mgm. Given 1 mgm. per kilogram.

TIME	ALUMINIUM TOTAL			PHOSPHORUS TOTAL			REMARKS
	Stomach	Small intestine	Large intestine	Stomach	Small intestine	Large intestine	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Mar. 26							Not fed
Mar. 27							15 grams $MgSO_4$
Mar. 29							
7:15							Amytal
10:30	0.000	0.000	0.032	0.25	0.78	0.27	Loops washed. Control sample
10:35							Injection begun
11:00							Injection finished
3:20	0.025	Lost	0.072	0.95	2.97	0.38	Died. Intestines appeared normal

Experiment J—control experiment

Dog, weight 14.0 kgm. Intravenous injection of physiological saline solution, 10 cc. per kilogram.

TIME	ALUMINIUM TOTAL			PHOSPHORUS TOTAL			REMARKS
	Stomach	Small intestine	Large intestine	Stomach	Small intestine	Large intestine	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
May 19							Not fed
May 20							Not fed
May 21							15 grams $MgSO_4$
May 22							
7:15							Amytal
9:50	0.000	0.000	0.000	0.70	1.02	1.00	Loops washed. Control samples
9:55							Injection begun
10:40							Injection finished
3:00 p.m.	0.000	0.000	0.000	1.59	3.40	1.19	Died. Intestinal tract appeared normal

DISCUSSION OF RESULTS. The fate of aluminium after intravenous injection of 1 or 2 mgm. per kilo in the dog is shown in these experiments. Its distribution to the various fluids of the body occurs promptly. Aluminium appears in the bile, lymph and urine in a few minutes after the injection. At the same time the amount in the blood tends to decrease

although fluctuations occur. The rate of disappearance is slow, the aluminium of the blood sometimes maintaining a fairly constant high level for a number of hours.

The rate of disappearance also varies from moment to moment, as does the rate of increase or decrease in the other fluids. This must depend on complex physical fluctuations such as circulatory rate and blood pressure.

The amount of aluminium appearing in the bile, lymph and urine is not always enough to account for that which has disappeared from the blood in the same time interval. This points to storage, temporary or permanent, of some of the injected aluminium. If we may suppose that a dog fasted for a day or so before the experiment is in "aluminium-equilibrium" we might expect that all the aluminium injected could be recovered if the experiment could be prolonged sufficiently.

Experiment F suggests that aluminium may be absorbed from the gastro-intestinal tract by the lymph, rather than through the blood since the blood throughout this experiment did not show aluminium, while the lymph did. We have no normal control sample on this animal, but from the results for the others we may assume that there was only a trace, if any, of aluminium in the normal lymph. Since the lymph from the thoracic duct was diverted this aluminium did not reach the blood. This is only one experiment, but the suggestion is very interesting, and conjectures as to its meaning lead to the question of the relation of aluminium to fat metabolism.

The last group of experiments shows another fact, that aluminium is excreted through the walls of the intestinal tract. These experiments are not to be regarded as quantitative, since exact measurements of the amount of the tissues used, and control time intervals were not attempted. This type of experiment could be made quantitative and information could be gained concerning the rate of the excretion of ions through the walls of the gastro-intestinal tract at various levels.

It is clearly shown that after intravenous injection of 1 mgm. per kilo of aluminium salts, aluminium is excreted through the walls of the stomach, the small and large intestine. This cannot be attributed to contamination, since all parts were carefully washed beforehand, and the bile excluded.

The fact that aluminium is in part eliminated through all portions of the gastro-intestinal tract may account for the difficulty of finding it in large quantities in the blood.

Determination of phosphorus was made because of an idea which has long held the approval of physiologists, although repeatedly discredited by careful investigation. This idea is that aluminium, if absorbed from the intestinal tract, will divert phosphorus from urinary to fecal excretion to form insoluble aluminium-phosphate, thus rendering the phosphorus

"unavailable" for metabolic uses. Modern physiology would see in such a diversion only another manifestation of the body's delicate equilibratory mechanism. It is, as we have seen, not yet proved that aluminium phosphate is formed, or that if formed it is insoluble in the gastro-intestinal tract. But it was desirable to put this idea to a direct test.

A comparison of results shows that, as closely as we may judge from such inexact experiments, as much phosphorus is excreted through the intestinal walls after an intravenous injection of physiological saline as after aluminium solutions. There is here no evidence that phosphorus excretion through the intestinal wall is increased by the presence of aluminium. These two are probably independent of each other. In general it would seem that more aluminium is excreted, per unit of area, through the walls of the small intestine than through the stomach or large intestine. The same seems to be true for phosphorus.

These experiments exemplify the physiological principle that the body maintains an exact ionic balance which may be disturbed for a short time by the sudden addition of an excessive amount of one ion, but the equilibrium with respect to that ion is adjusted promptly by distributing it through the body and by prompt excretion.

One thing worthy of note is the decrease in urinary excretion after the injection of aluminium salts. While we have no data of the normal excretion rate for these animals it is obvious that an excretion of 22.1 cc. in seven hours (expt. B) and 22.7 cc. in six and one-half hours (expt. D) is very low for dogs of this size, especially as 5 or 10 cc. per kilo of fluid had been injected. In some cases no urine was obtained after the injection. This cannot be attributed to low blood pressure as in only one case was there any evidence of shock. In one case when complete anuria occurred, sections of the kidney were made. These showed nothing abnormal. We are therefore left to question the effect of the aluminium injection upon the kidney.

CONCLUSIONS

1. After the intravenous injection of aluminium salts in doses of 1 or 2 mgm. of aluminium per kilo in dogs, the metal appears promptly in the bile, lymph and urine.

2. As far as may be judged, this increase in these fluids does not account for the aluminium which has disappeared from the blood. This points to storage in the tissues.

3. Aluminium is absorbed by the lymph when aluminium-rich food is fed to dogs.

4. Aluminium is excreted through the walls of the gastro-intestinal tract at all levels. It is suggested that this gastro-intestinal elimination of aluminium may account for the failure to demonstrate large quantities of the metal in the blood after ingestion of aluminium-rich food.

5. The aluminium and phosphorus excretion through the walls of the alimentary canal are independent of each other.

BIBLIOGRAPHY

(1) Steel, M. This Journal, 1911, xxviii, 94.
(2) Myers, V. C., and D. B. Morrison. Journ. Biol. Chem., 1928, lviii, 615.
(3) Underhill, F. P. and F. I. Peterman. This Journal, 1929, xc, 15.
(4) Underhill, F. P. and F. I. Peterman. This Journal, 1929, xc, 1.
(5) Bell, R. D. and E. A. Doisy. Journ. Biol. Chem., 1920, xliv, 55.

Am. J Physiol 90: 67-71, (1929)

STUDIES IN THE METABOLISM OF ALUMINIUM

VI. The Occurrence of Aluminium in Human Liver and Kidney

FRANK P. UNDERHILL, F. I. PETERMAN, E. G. GROSS and A. C. KRAUSE

From the Department of Pharmacology and Toxicology, Yale University, New Haven, Conn.

Received for publication April 19, 1929

There are very few reports of the aluminium content of human tissues. Gonnerman (1) (2) presented analyses of a limited number of tissues from animals and man. The large amounts found are probably due to the fact that ashing was carried out in porcelain crucibles, which we have found to give high results. Keilholz (3) shortly afterwards reported no aluminium found in several human tissues analyzed. The method used, that of Atack (4), we have found to give low results. The reasons for this are discussed elsewhere (5). Myers and Mull (6) have recently published some results of analyses of autopsy material. Since we have not tested their method we are not prepared to evaluate their results. However, on theoretical grounds it is quite possible that aluminium may be lost during the manipulations. Moreover their dye is not as sensitive an indicator for aluminium as is alizarine.

That human tissues usually contain small traces of aluminium can no longer be doubted. But the relation between the aluminium content of tissues and age, sex, environment and state of metabolism is still to be determined. In the analyses presented below an indication is given as to the direction such an investigation may take.

The tissues analyzed were obtained through the generous coöperation of pathologists all over the United States. While analyses of only a part of the material sent is presented here, we take this opportunity of thanking all those whose coöperation made this study possible.

The livers and kidneys, (preserved with 95 per cent alcohol, occasionally with formalin), obtained at autopsy, were expressed to us, usually in glass containers. Tissues were dried and analyzed as previously described (5). The preserving fluid was also analyzed, and usually found to contain an inconsiderable amount of aluminium.

Results. The results are tabulated below. Analyses for the aluminium content of the liver from twenty-two human subjects are presented, and for kidneys of twenty-one of these. Ages range from 5 to 73 years. Six widely separated localities are represented.

DISCUSSION. Table 1 is a summary of the analyses for the aluminium content of the liver and kidney. It will be seen that the aluminium content ranges from 0.17 to 1.17 mgm. of aluminium per 100 grams or a total of

TABLE 1

The aluminium content of the human liver and kidney

Al in milligrams per 100 grams

PLACE	AUTOPSY NUMBER	AGE	SEX	DIAGNOSIS	LIVER			KIDNEY		
					Weight	Al/100 grams	Total Al	Weight	Al/100 grams	Total Al
					grams			grams		
Boston, Mass.	A-24-58	27	M.	Tuberculous meningitis	1,280	0.31	5.65	300	0.43	1.28
	A-24-59	39	M.	Brain abscess	1,460	0.33	4.85	310	0.23	0.71
	A-24-62	49	M.	Brain tumor, lobar pneumonia	1,850	0.27	5.05	410	0.21	0.83
	A-24-63	38	M.	Pneumonia	1,500	0.41	6.21	210	0.69	1.44
Ann Arbor, Mich.	A-190-AB	44	F.	Diabetes	1,905	1.17	22.27	220*	0.64	1.47*
	A-192-AB	67	M.	Peritonitis	1,330	0.80	10.68	162*	0.74	0.45*
	A-193-AB	70	M.	Cerebro-spinal meningitis	1,630	0.84	13.70	145*	0.87	1.26*
	A-195-AB	Adult	F.	Carcinoma	1,360	0.53	7.17	205*	0.38	0.78*
Birmingham, Ala.		73	M.	Scalp injuries, pneumonia	1,102	0.38	4.24	187	0.78	1.46
Cleveland, Ohio	2914	63	M.†	Pneumonia	1,750	0.30	5.24	250	0.31	1.01
	2915	45	M.†	Pulmonary tuberculosis	1,825	0.21	3.89	450	0.26	1.19
	2917	35	F.	Septicemia	1,850	0.53	9.50	325	0.17	0.44
	2918	21	F.	Pulmonary tuberculosis	1,750	0.21	3.71	300	0.22	0.66
	2919	45	M.	Tuberculous meningitis	1,425	0.21	2.97	325	0.28	0.90
New Haven, Conn.	898	65	M.	Arterio-sclerosis	1,450	0.26	3.77	285	0.19	0.53
	901	46	F.	Carcinoma of stomach	1,510	0.27	4.09	300	0.13	0.39
	908	32	M.	Pulmonary tuberculosis	1,590	0.34	5.40	280	0.20	0.56
	911	5	F.	Acute myocarditis	540	0.19	1.01	105	0.15	0.16
	912	43	M.	Peritonitis	1,709	0.39	6.65	285	0.30	0.85
Augusta, Ga.	24-38	Adult	M.†	Gun shot wounds	1,250	0.72	9.02	365	0.71	2.59
	24-45	21	F.†	Typhoid	1,905	0.60	11.41	385	0.61	2.34
	24-47	45	F.†	Nephritis	1,335	0.17	2.24	230		

* One kidney only. † Negro.

2.24 to 22.27 mgm. for the whole organ, if we except the only child in the series, having a total content of 1.01 mgm. The kidney varies from 0.13 to 0.87 mgm. per 100 grams with a total of 0.39 to 2.59 mgm. if we again

except the child with a value of 0.16. There is no absolute agreement between the amount found in the liver and kidney, but in general a low value is found for the kidney when a low value is present for the liver. The opposite tendency is noted, when the liver value is high the kidney value is also likely to be high. That this is not always the case will appear by reference to no. A-190-AB, in which the highest liver value is found. The kidney value is high but not the highest. In no. 901 the lowest kidney content is accompanied by a low but not the lowest liver content. The only case that can be considered normal is no. 24-38, an adult colored male dying from gun shot wounds. Tissues from this case show what seems to be a moderately high aluminium content for both liver and kidney.

Table 2 shows the distribution of aluminium in the tissues with respect to locality. In this table the average value is given for tissues from particular places. It will be noted that livers of cases from Ann Arbor,

TABLE 2

Distribution of aluminium in liver and kidney related to locality

PLACE	NUMBER OF SPECIMENS	AVERAGE Al IN LIVER PER 100 GRAMS	AVERAGE Al IN KIDNEY PER 100 GRAMS
Boston, Mass.	4	0.33	0.39
Ann Arbor, Mich.	4	0.83	0.66
Birmingham, Ala.	1	0.38	0.78
Cleveland, Ohio	5	0.29	0.24
New Haven, Conn.	5	0.29	0.19
Augusta, Ga.	3	0.50	(2) 0.66
Average		0.43	0.40

Mich., show the highest aluminium content, the kidney from Birmingham, Ala., the highest kidney value, although it is not a fair comparison to include this latter result in a table of averages. The kidneys from Ann Arbor also show a high aluminium content. Tissues from New Haven, Conn., and Cleveland, O., show the lowest aluminium content. Tissues from Boston, Mass., have a moderately low aluminium content.

The consistently high values for tissues from Michigan, and the consistently low ones from Connecticut are worthy of notice, as even cases of approximately the same age, selected from these two groups show this wide variation. (Compare no. A-190-AB with no. 901 and no. 910, and no. A-192-AB with no. 898.) These differences are probably not accidental, and could conceivably be traced to some factor in the environment, such as diet, water supply or type of soil.

When the results are tabulated according to age (see table 3) it is seen that the general tendency is for the aluminium content to increase with

TABLE 3

The relation of aluminium content to age and disease

AGE	SEX	LOCALITY	LIVER Al/100 GRAMS	KIDNEY Al/100 GRAMS	AVERAGE LIVER	AVERAGE KIDNEY	OTHER PERTINENT PATHOLOGIC DETAILS
5	F.	Conn.	0.19	0.15	0.19	0.15	
21	F.	Ohio	0.21	0.22	0.38	0.42	Chronic passive congestion of liver
21	F.*	Ga.	0.60	0.61			Ruptured ectopic hemorrhage
27	M.	Mass.	0.31	0.43			Tuberculosis in liver and kidneys
32	M.	Conn.	0.34	0.20	0.40	0.41	
35	F.	Ohio	0.53	0.17			Cloudy swelling and congestion of liver and kidney
38	M.	Mass.	0.41	0.69			Acute suppurative nephritis
39	M.	Mass.	0.33	0.23			? Pyelitis
43	M.	Conn.	0.39	0.30	0.40	0.30	
44	F.	Mich.	1.17	0.64			Fatty liver
45	M.*	Ohio	0.21	0.26			Caseous tuberculosis and congestion of liver
45	M.	Ohio	0.21	0.28			Bilateral pyelonephritis
45	F.*	Ga.	0.17				Congestion of kidneys
46	F.	Conn.	0.27	0.13			Anemia
49	M.	Mass.	0.27	0.21			
63	M.*	Ohio	0.30	0.31	0.47	0.41	Cloudy swelling of liver and kidney. Mild chronic interstitial nephritis
65	M.	Conn.	0.26	0.17			Generalized arterio-sclerosis
67	M.	Mich.	0.80	0.74			Passive congestion, atrophy and parenchymatous degeneration of all organs
70	M.	Mich.	0.84	0.87	0.61	0.82	Multiple abscesses of liver. Chronic degenerative parenchymatous nephritis. Arterio-sclerosis, adenoma of kidney
73	M.	Ala.	0.38	0.78			Liver small, moderate chronic passive congestion
Adult	M.*	Ga.	0.72	0.71			All organs sound
Adult	F.	Mich.	0.53	0.38			Generalized arterio-sclerosis, atrophy and parenchymatous degeneration of all organs

* Negro.

advancing age. This trend has been indicated for dogs in a previous paper (7).

When one tries to find a relationship between the state of the tissue analyzed and the aluminium content there is one outstanding result (see table 3). The liver showing much the highest aluminium content (A-190-AB) is described in the autopsy report as a "fatty liver." It has previously been suggested that aluminium may be in some way related to fat metabolism (8). While this single result may be due to other unknown factors it again raises the question of a relation between these two substances.

Five cases in which tuberculous infection was a prominent feature show a low aluminium content for both liver and kidney, the age range being 21 to 45 years, the value for liver 0.21 to 0.34 mgm. per 100 grams and for kidney 0.22 to 0.43 mgm. However, these all came from districts showing low aluminium content. It is interesting that two cases of the same age, from the same locality show the same aluminium content in both liver and kidney (see no. 2915 and no. 2919, Cleveland, O.).

There is no apparent relationship between arterio-sclerosis and aluminium content, since one case (no. 898, New Haven, Conn.) shows a low aluminium content and another (no. A-193-AB, Ann Arbor, Mich.) shows a high aluminium content.

SUMMARY

1. The aluminium content of livers and kidneys from human autopsy material is reported.

2. The range found for liver is from 0.17 to 1.17 mgm. per 100 grams, for kidney from 0.13 to 0.87 mgm. per 100 grams.

3. The significance of some of the findings is discussed.

BIBLIOGRAPHY

(1) Gonnerman, M. Biochem. Zeitschr., 1918, xxviii, 401.
(2) Gonnerman, M. Zeitschr. physiol. Chem., 1920, cxi, 32.
(3) Keilholz, A. Pharm. Weekbl., 1921, lviii, 1482.
(4) Atack, F. W. Journ. Soc. Chem. Ind., 1915, xxxiv, 936.
(5) Underhill, F. P. and F. I. Peterman. This Journal, 1929, xc, 1.
(6) Myers, V. C. and J. W. Mull. Journ. Biol. Chem., 1928, lviii, 625.
(7) Underhill, F. P. and F. I. Peterman. This Journal, 1929, xc, 62.
(8) Underhill, F. P., F. I. Peterman and S. L. Steel. This Journal, 1929, xc, 52.

The Pharmacopeia of the United States of America

(*The United States Pharmacopeia*)

Eighteenth Revision

By authority of
The United States Pharmacopeial Convention, Inc.,
meeting at Washington, D. C., March 29 and 30, 1960

Prepared by the
Committee of Revision and Published by the Board of Trustees

Official from September 1, 1970

4630 MONTGOMERY AVENUE
BETHESDA, MD. 20014

Heavy metals, page 897—Dissolve 5 g. in 10 ml. of diluted hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 ml.: the heavy metals limit is 5 parts per million.

Assay—Transfer about 25 g., accurately weighed, of Aluminum Hydroxide Gel to a beaker, add 15 ml. of hydrochloric acid, and heat gently until solution is complete. Cool, transfer to a 500-ml. volumetric flask, dilute with water to volume, and mix. Pipet 20 ml. of this solution into a 250-ml. beaker, and add, in the order named and with continuous stirring, 25.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate and 20 ml. of acetic acid–ammonium acetate buffer T.S., then heat the solution near the boiling point for 5 minutes. Cool, and add 50 ml. of alcohol and 2 ml. of dithizone T.S. Titrate the solution with 0.05 *M* zinc sulfate until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 ml. of water for the sample, and make any necessary correction. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate consumed is equivalent to 2.549 mg. of Al_2O_3.

Packaging and storage—Preserve in tight containers, and avoid freezing.

CATEGORY: Antacid.

USUAL DOSE: 15 ml. four to six times a day.

USUAL DOSE RANGE: 5 to 30 ml. up to twelve times daily.

Dried Aluminum Hydroxide Gel

Dried Aluminum Hydroxide Gel yields not less than 50.0 percent of aluminum oxide (Al_2O_3).

Description: White, odorless, tasteless, amorphous powder.

Solubility: Insoluble in water and in alcohol; soluble in dilute mineral acids and in solutions of fixed alkali hydroxides.

Identification—Dissolve 500 mg. in 10 ml. of diluted hydrochloric acid, with gentle warming: the solution responds to the tests for *Aluminum*, page 891.

Reaction—Agitate 1 g. with 25 ml. of water, and filter: the filtrate is neutral to litmus.

Acid-consuming capacity—Weigh accurately 200 to 250 mg., and transfer to a glass-stoppered, 250-ml. flask. Add 100.0 ml. of 0.1 *N* hydrochloric acid, and shake the mixture continuously at 37° for 1 hour. To 50.0 ml. of the solution add bromophenol blue T.S., and titrate the excess acid with 0.1 *N* sodium hydroxide: the volume of 0.1 *N* acid consumed is not less than 250 ml. for each g. of Dried Aluminum Hydroxide Gel.

Chloride, page 895—Dissolve 1 g. in 30 ml. of diluted nitric acid, heat to boiling, add water to make 100 ml., and filter: a 5-ml. portion of the filtrate, diluted with an equal volume of water, shows no more chloride than corresponds to 0.6 ml. of 0.02 *N* hydrochloric acid (0.85 percent).

Sulfate, page 895—Dissolve 330 mg. in 15 ml. of diluted hydrochloric acid, heat to boiling, add water to make 250 ml., and filter: a 25-ml. portion of the filtrate shows no more sulfate than corresponds to 0.2 ml. of 0.02 *N* sulfuric acid (0.6 percent).

Arsenic, page 894—Dissolve 2.5 g. in 20 ml. of dilute sulfuric acid (1 in 5), and dilute with water to 55 ml.: the resulting solution meets the requirements of the test, the addition of 20 ml. of dilute sulfuric acid (1 in 5) specified under *Procedure* being omitted (8 parts per million).

Heavy metals, page 897—Dissolve 400 mg. in 10 ml. of diluted hydrochloric acid with the aid of heat, filter if necessary, and dilute with water to 25 ml.: the heavy metals limit is 60 parts per million.

Assay—Weigh accurately about 2 g. of Dried Aluminum Hydroxide Gel, and dissolve in 15 ml. of hydrochloric acid, with the aid of heat. Cool, transfer to a 500-ml. volumetric flask, dilute with water to volume, and mix. Pipet 20 ml. of this solution into a 250-ml. beaker, and add, in the order named and with continuous stirring, 25.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate and 20 ml. of acetic acid–ammonium acetate buffer T.S., then heat the solution near the boiling point for 5 minutes. Cool, and add 50 ml. of alcohol and 2 ml. of dithizone T.S. Titrate the solution with 0.05 *M* zinc sulfate to a bright rose-pink color. Perform a blank determination, substituting 20 ml. of water for the sample solution, and make any necessary correction. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 2.549 mg. of Al_2O_3.

Packaging and storage—Preserve in tight containers.

color is obtained. Heat to boiling, and again neutralize. Add 150 ml. of glycerin to neutralized solution, and titrate with 0.5 *N* sodium hydroxide. Perform a blank termination in a similar manner. From the volume of 0.5 *N* sodium hydroxide used the addition of the glycerin, subtract the volume used in the blank. Each ml. of 0. sodium hydroxide is equivalent to 30.92 mg. of H_3BO_3.

Assay for aluminum oxide—Pipet 25 ml. of Aluminum Acetate Solution into a 250 volumetric flask, add 5 ml. of hydrochloric acid, dilute with water to volume, and Pipet 25 ml. of this dilution into a 250-ml. beaker, and add, in the order named and continuous stirring, 25.0 ml. of 0.05 *M* disodium ethylenediaminetetraacetate and 20 of acetic acid–ammonium acetate buffer T.S., then heat the solution near the bo point for 5 minutes. Cool, and add 50 ml. of alcohol and 2 ml. of dithizone T.S. Ti the solution with 0.05 *M* zinc sulfate until the color changes from green-violet to rose-p Perform a blank determination, substituting water for the sample, and make any ne sary correction. Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equiva to 2.549 mg. of Al_2O_3.

Assay for acetic acid—Pipet 20 ml. of Aluminum Acetate Solution into a Kjeldahl containing a mixture of 20 ml. of phosphoric acid and 150 ml. of water. Connec flask to a condenser, the delivery tube from which dips beneath the surface of 50.0 m 0.5 *N* sodium hydroxide contained in a receiving flask. Distil about 160 ml., then rem the delivery tube from below the surface of the liquid, allow the distilling flask to cool, 50 ml. of water, and distil an additional 40 to 45 ml. into the receiving flask. phenolphthalein T.S. to the distillate, and titrate the excess 0.5 *N* sodium hydroxide 0.5 *N* sulfuric acid. Each ml. of 0.5 *N* sodium hydroxide is equivalent to 30.03 mg $C_2H_4O_2$.

Packaging and storage—Preserve in tight containers.

CATEGORY: Astringent.

FOR EXTERNAL USE: Topically to the skin and mucous membranes, dilu with 10 to 40 parts of water in a wet dressing.

Aluminum Hydroxide Gel

Aluminum Hydroxide Gel is a suspension, each 100 g. of which contains equivalent of not less than 3.6 g. and not more than 4.4 g. of aluminum o (Al_2O_3), in the form of aluminum hydroxide and hydrated oxide. It may c tain peppermint oil, glycerin, sorbitol, sucrose, saccharin, or other suit flavors, and it may contain suitable antimicrobial agents in a total amo not exceeding 0.5 percent.

Description: White, viscous suspension, from which small amounts of clear liquid separate on standing.

Identification—A solution in hydrochloric acid responds to the tests for *Aluminum*, 891.

Microbial limits, page 846—Its total aerobic microbial count does not exceed 100 per and it meets the requirements of the test for absence of *Escherichia coli*.

pH, page 938: between 5.5 and 8.0, determined potentiometrically.

Acid-consuming capacity—Transfer about 1.5 ml. of the well-shaken Gel to a tared, g stoppered, 125-ml. flask, and weigh. Add 50.0 ml. of 0.1 *N* hydrochloric acid, and s the mixture continuously at 37° for 1 hour. Then add bromophenol blue T.S., and ti the excess acid with 0.1 *N* sodium hydroxide. The volume of 0.1 *N* acid consumed i less than 12.5 ml. and not more than 25.0 ml. for each g. of the Gel.

Chloride—Transfer 10 g. to a porcelain dish. Add 0.1 ml. of potassium chromate T.S. 25 ml. of water. Stir, and add 0.1 *N* silver nitrate until a faint persistent pink col obtained: not more than 8 ml. of 0.1 *N* silver nitrate is required (0.28 percent).

Sulfate, page 895—Add 5 ml. of diluted hydrochloric acid to 5 g. of it, and heat to di the sample. Cool, dilute with water to 250 ml., and filter if necessary: a 20-ml. po of the filtrate shows no more sulfate than corresponds to 0.2 ml. of 0.02 *N* sulfuric (500 parts per million).

Arsenic, page 894—Dissolve 2.5 g. in 20 ml. of dilute sulfuric acid (1 in 5), and add 35 m water: the resulting solution meets the requirements of the test, the addition of 20 of dilute sulfuric acid (1 in 5) specified under *Procedure* being omitted (0.6 part per mil

CATEGORY: Antacid.

USUAL DOSE: The equivalent of 300 mg. of aluminum hydroxide four to six times a day.

USUAL DOSE RANGE: The equivalent of 300 mg. to 5 grams of aluminum hydroxide daily.

Dried Aluminum Hydroxide Gel Tablets

Dried Aluminum Hydroxide Gel Tablets contain an amount of aluminum oxide (Al_2O_3) equivalent to not less than 62.0 percent and not more than 72.0 percent of the labeled amount of aluminum hydroxide.

Identification—Digest a portion of finely powdered Tablets, equivalent to about 500 mg. of dried aluminum hydroxide gel, with 10 ml. of diluted hydrochloric acid with gentle warming, and filter: the filtrate responds to the tests for *Aluminum*, page 891.

Disintegration, page 932: 30 minutes.

Weight variation, page 950: meet the requirements for *Tablets*.

Assay—Weigh and finely powder not less than 20 Dried Aluminum Hydroxide Gel Tablets. Weigh accurately a portion of the powder, equivalent to about 2 g. of dried aluminum hydroxide gel, add 15 ml. of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 ml., mix, and filter quantitatively into a 500-ml. volumetric flask, washing the filter with water. Proceed as directed in the *Assay* under *Dried Aluminum Hydroxide Gel*, page 27, beginning with "dilute with water to volume." Each ml. of 0.05 *M* disodium ethylenediaminetetraacetate is equivalent to 3.900 mg. of $Al(OH)_3$.

Packaging and storage—Preserve in well-closed containers.

Tablets available—Tablets usually available contain the following amounts of aluminum hydroxide: 300, 500, and 600 mg.

CATEGORY and DOSE: See *Dried Aluminum Hydroxide Gel*.

Aluminum Monostearate

Aluminum Monostearate is a compound of aluminum with a mixture of solid organic acids obtained from fats, and consists chiefly of variable proportions of aluminum monostearate and aluminum monopalmitate. It contains the equivalent of not less than 14.5 percent and not more than 16.0 percent of Al_2O_3.

Description: Fine, white to yellowish white, bulky powder, having a faint, characteristic odor.

Solubility: Insoluble in water, in alcohol, and in ether.

Identification—

A: Heat 1 g. with a mixture of 25 ml. of water and 5 ml. of hydrochloric acid for 1 hour, replacing the water as it evaporates: fatty acids are liberated, floating as an oily layer on the surface of the liquid, and the water layer responds to the tests for *Aluminum*, page 891.

B: Mix 25 g. with 200 ml. of ether in a separator, add 150 ml. of diluted hydrochloric acid, and shake the mixture vigorously until the layers separate cleanly on standing. Remove the water layer, and wash the ether layer with three 30-ml. portions of water. Transfer the ether layer to a small beaker, warm on a steam bath until the ether has evaporated and the fatty acids are clear, and dry the acids at 105° for 20 minutes: the solidification temperature (see page 905) of the fatty acids is not below 54°.

Loss on drying, page 935—Dry it at 80° for 16 hours: it loses not more than 2.0 percent of its weight.

Arsenic, page 894—To 5 g. add 12.5 ml. of hydrochloric acid and 0.5 ml. of bromine T.S., and heat on a steam bath until a transparent layer of melted fatty acid forms. Add 50

1132

HYPERALUMINÆMIA FROM ALUMINIUM RESINS

SIR,—Professor Berlyne and his colleagues (Sept. 5, p. 494) report that a third of their uræmic patients taking oral aluminium-cycle resins or aluminium hydroxide had traces of aluminium detectable in their serum. From this they conclude that aluminium toxicity is a real danger of such treatment. To us this finding suggests that the aluminium ion is relatively non-toxic, otherwise the enormous amounts of the element consumed by mankind over the ages would by now have led to recognition of a syndrome of aluminium intoxication.

Aluminium is the third most abundant element on the surface of the earth, and is so widespread in distribution that inevitably most people consume it unintentionally from time to time. It is one of the main constituents of clay, and is consumed as such in various forms of pica and as medicinal kaolin. Aluminium hydroxide was introduced in the treatment of peptic ulcer in 1924,[1] and has since been used by many millions of patients, particularly in the United States, where it is the main ingredient of the most popular proprietary antacids. Almost thirty years have passed since aluminium hydroxide was introduced for the control of hyperphosphataemia in uraemia,[2] and since then it has been recommended by many experts on renal failure,[3,4] not least by Professor Berlyne himself.[5] We were unaware of any report of systemic toxicity from this widespread use when we suggested that oral aluminium resin would be useful in simultaneously controlling the hyperkalæmia and hyperphosphataemia of renal failure,[6] and to our knowledge no reports of toxicity have appeared since, except for the ill-effects which can result from excess phosphate depletion.[7]

Professor Berlyne and his colleagues disregard, or are unaware of, this large body of evidence showing the safety of aluminium salts, and on the basis of their serum values alone they suggest that the drug should be abandoned—though its ability to lower plasma-phosphate, and hence reduce the danger of soft-tissue calcification in uraemia, is not in question. A blind eye is turned on Professor Berlyne's own observations on the dangers of a high plasma Ca × P product [8,9]; for calcium resin, which may increase this product further,[10,11] is advocated as the best form of resin.

1. Guillermin, R. *Revue méd. Suisse romande*, 1924, 44, 243.
2. Freeman, S., Freeman, W. M. C. *Am. J. Dis. Child.* 1931, 51, 981.
3. Merrill, J. P. The Treatment of Renal Failure. London, 1965.
4. Pendras, J. P., Erickson, R. V. *Ann. intern. Med.* 1966, 64, 302.
5. Mallick, N. P., Berlyne, G. M. *Lancet*, 1966, ii, 1316.
6. Chugh, K. S., Swales, J. D., Brown, G. L., Wrong, O. M. *ibid.* p. 952.
7. Lotz, M., Zisman, E., Bartter, F. C. *New Engl. J. Med.* 1968, 278, 409.
8. Berlyne, G. M., Shaw, A. B. *Lancet*, 1967, i, 4.
9. Berlyne, G. M. *ibid.* 1968, ii, 396.
10. Parfitt, A. M., Gingell, J. C., Chisholm, G. D. *ibid.* p. 948.
11. Sevitt, L. A., Wrong, O. M. *ibid.* p. 950.

1131

We look forward to the further studies promised by Professor Berlyne and his colleagues, and would be particularly interested to know whether uræmic patients have higher serum-aluminium concentrations than normal subjects given the same dose of aluminium. (They imply that this is so, but do not give the data, and it is quite possible that aluminium absorption is reduced in renal failure because of high phosphate concentrations in the intestine.) Even this finding would not establish the toxicity of aluminium, but toxicity would be suggested if known differences in the incidence of uræmic complications (e.g., neuropathy, myopathy) between different renal centres could be related to the use of aluminium salts. Until more evidence of this sort is produced we will feel justified in continuing to use aluminium preparations in the treatment of hyperphosphataemia.

Departments of Medicine,
Dundee University and
Manchester Royal Infirmary.

O. M. WRONG
J. D. SWALES.

Biochem. Z.
265:169-180, 1933

Absorption of Aluminum Compounds in the Organism, Taking into Account the Normal Aluminum Content of Animal Tissue.

by

J. Wührer

From: Pharmacological Laboratory of the National Health Office.

Received: July 29, 1933.

The question of whether the continuous ingestion of small quantities of aluminum compounds with food can cause any kind of damage to health has been the subject of thorough and prolonged study, and on the basis of the results, has been answered in the negative (1). The question has arisen once more and become the subject of new experimental studies (2) owing to the claims publicized in recent years that the use of aluminum eating, drinking and cooking utensils could be hazardous to health.

An important factor in assessing the problem of the pharmacological effect of metal compounds in the animal organism is to establish the extent to which the metal is absorbed by the gastrointestinal tract, the course of the excretion of the metal and whether the metal is stored in organs or tissues. With regard to aluminum there are only a few recent comprehensive studies on this subject.

Myers et al. (3) and Peterman and Underhill (4) found that the administration of relatively large quantities of aluminum per os had virtually no effect on the normal aluminum content of the organs and tissues of dogs and rats. A small amount of aluminum was detected in normal animal and human organs and tissues which, for instance in the liver, ranged between 0.15 and 0.66 mg and in the blood between 0.06 and 0.36 mg fresh organic material. Upon prolonged administration of aluminum compounds in the form of biscuits containing aluminum baking powder to the animals no storage of aluminum could be detected in the organs etc. This replaced the conclusions of early studies by Steel (5), Kahn (6) and Balls (7) according to which considerable quantities of aluminum are absorbed.

K. MacKenzie (8) has further established on a large number of animals (rats and pigs) that the excretion of the aluminum taken per os remains entirely limited to the gastro-intestinal tract and no detectable absorption takes place. Steudel (l.c.) has assumed that the aluminum salt that has found its way into the digestive tract either is not absorbed at all, or, like iron, after temporary absorption in the upper segments of the small intestine, it is then excreted again without residue by the large intestine (9).

In addition to the authors cited, the occurrence of aluminum in normal animal organs and tissues has recently been

established by Kahlenberg and Closs (10), Underhill, Peterman, Gross and Krause (11), Massatsch (l.c.), K.H. Lehmann (12) and S.J. Lewis (13)(14). The results of the studies of Gonnermann (15) on the aluminum content of human organs - values of 0.2 to 3.4 % of dry substance were found - cannot be regarded as correct in view of the doubtful nature of the method of determination, and according to all existing investigations and our own experiments, they are no longer valid.

In earlier, as yet unpublished studies of our laboratory on the pharmacological effect of aluminum in dogs, a loss of appetite was observed in some cases when aluminum salts with an acid reaction were administered in the food for several weeks (potash alum: 3.2 to 6.4 g, aluminum sulfate: 4.5 g and aluminum acetate solution: corresponding to 0.125 to 0.25 Al_2O_3 per animal). No aluminum could be detected in the urine of the animals, while in the bowel excretion the total quantity of the aluminum administered was recovered. It was concluded from this that even upon prolonged administration of large quantities of aluminum salts to dogs, no harmful absorption effect could be observed and the loss of appetite observed could be attributed to the local astringent, in some cases corrosive effect of these large quantities of strongly hydrolyzing aluminum salts.

The incorporation of large quantities of soluble aluminum salts that cause irritation of mucosa cannot be compared,

when making a pharmacological assessment, with the ingestion of small quantities of aluminum compounds with food. It was therefore necessary to determine whether with very prolonged administration of small quantities of a readily soluble aluminum salt with the daily food on the one hand, and a similar administration of large quantities of an aluminum compound that exerts no irritating effect on the mucosa on the other, any absorption and storage of aluminum can be observed in the organs and tissues. For the experiments described below aluminum chloride ($AlCl_3+6H_2O$) in gelatin capsules was used on the one hand and on the other freshly precipitated aluminum hydroxide (which shows strong solubility in weakly acid fluids), in the food.

In order to obtain the most homogeneous form possible of the aluminum hydroxide to be fed to the animals, a solution of crystallized aluminum sulfate (60 g) was precipitated at boiling heat with a small excess of ammonia, immediately sucked off still hot and washed hot. The aluminum hydroxide mass was mixed with water, heated to boiling, agitated for c. 2 hours on the agitator and poured through a fine-mesh sieve. The emulsion-like suspension was then filled up in a measuring flask to 1000 cc with water. 100 cc of the suspension, whose Al_2O_3 content was determined, was mixed in with the daily food (cooked rice, dried or fresh cooked meat) and given to the dogs. The food mixed with the suspension was

generally consumed by the animals with no left-overs.

Five dogs were fed in this manner uniformly every day for months, initially with smaller quantities (150, 300, 600 mg successively) and during the second period with suspensions containing an average of 1000 mg aluminum oxide (16) (see Table I).

The dogs (nos. 1-5) received a total of 190, 210, 225, 292 and 337 g aluminum oxide in the manner described. The aluminum chloride ($AlCl_2$-$6H_2O$) was administered to two dogs in solid form in gelatin capsules in a dose of 300 mg aluminum chloride per day, corresponding to 33 mg aluminum, in the morning immediately before the usual feeding time (see Table II).

Next, in single experiments on dogs and humans, the urine and feces were examined for the presence of aluminum after the administration of a single dose of an aluminum hydroxide suspension containing c. 1000 mg Al_2O_3, in some cases after the lapse of several hours, in others after several days. In addition, two experimental subjects (the author and Dr. Kärber) ingested a suspension containing c. 1000 mg Al_3O_3 daily for 17 days. One of the two subjects (Dr. Kärber) continued the experiment with a daily ingestion of suspension containing c. 500 mg Al_2O_3 for a period of 70 days.

I. Determination of aluminum in organic material

Of the organs removed from the sacrificed animals 100 g, or if the organ weighed less, the entire organ was used for analysis. To shorten the process of analysis and as far as

possible to exclude errors due to the addition of larger quantities of reagents (acids) during mineralization, we refrained from wet destruction of the organs, and since there is no risk of volatile loss of aluminum during ashing and careful glowing, the organs were ashed after prior drying in quartz cups over a free flame with drop-by-drop addition of small quantities of concentrated nitric acid (3 to 5 cc). Poorly combustible carbon was extracted with water, burnt, and the extraction fluid added to the ash. The same procedure was used with the stools. The urine was concentrated, the organic substance destroyed with smoking nitric acid and ashed in a quartz cup. The ash was dissolved in 10 to 15 cc 25% hydrochloric acid in a platinum or quartz cup while heating, and if necessary filtered off from the usually very small insoluble residue made up of silicic acid. In cases in which small quantities of copper could be expected (particularly in the liver), the hydrochloric acid solution was suitably diluted, precipitated with hydrogen sulfide, left to stand for several hours, filtered and concentrated down to c. 50 cc (17). The solution of ash was neutralized with ammonia up to a still barely acid reaction (phenophthalein as indicator), after which 1.8 cc hydrochloric acid (specific gravity 1.19) was added, diluted to c. 400 cc and successively 10 cc 10% disodiumphosphate solution, 25 cc 20% sodium thiosulfate solution and 7.5 cc 30% acetic acid were added.

If a permanent precipitation took place following the addition of sodium phosphate, as for instance in the case of material with a relatively high iron content (blood, liver), a further 1.8 cc hydrochloric acid and 25 cc sodium thiosulfate was added. The solution, becoming cloudy from incipient sulfur precipitation, was heated for 30 min to boiling, replacing the evaporated water, and after allowing the precipitate consisting of sulfur and aluminum phosphate to settle, it was filtered off, washed hot and the precipitate together with the filter was dried in the platinum crucible, ashed, glowed and weighed. Thereafter, even in the presence of iron and calcium, it is possible to precipitate the aluminum as phosphate without at the same time precipitating iron and calcium.

In blank experiments with aluminum sulfate solution (1.23 g $Al_2(SO_4)_3+18H_2O$ dissolved in 1 liter water) the following values were found. The quantities below were added to 20 cc distilled water:

Al added in mg
Found

Zugesetzt Al in mg . .	10,0	5,0	2,0	1,5	1,0	0,5	0,4	0,3	0,2	0,1
Gefunden „ „ „ . . .	9,9	4,95	1,9	1,5	0,99	0,5	0,44	0,27	0,22	0,15

Without addition of aluminum an average blank value of 0.3 mg was found, which, calculated as aluminum, gives 0.06 mg aluminum and was deducted from all the values determined.

The quantities below were added to crushed organs (per 100 g muscle, liver, intestine):

Al added per mg	Zugesetzt Al in mg . .	5,0	3,0	2,0	2,0	1,5	1,0	0,6	0.35	0.2
Found	Gefunden „ „ „ . .	4,95	3,0	2,04	2,1	1.37	0,85	0,69	0,3	0.2 *

* Die ohne Aluminiumzusatz erhaltenen Werte (s. Tabelle) sind in Abzug gebracht.

* The values obtained without added aluminum (see table) were deducted.

Thereafter the method also allows the detection of very small quantities of aluminum in organic material with a satisfactory degree of accuracy and a limit of error of c. ±20%. The colorimetric method reported by Underhill and Peterman (l.c.) yielded values in control experiments that were in good accord with the values obtained by weight analysis.

II. Results

1. Long-term experiments.

The aluminum values determined in organs etc. are shown in Table I.

In the daily urine (24 hours)(18) of animals fed in long-term experiments with aluminum hydroxide and aluminum chloride no aluminum or only traces (up to 0.15 mg Al) could be detected, just as in the daily urine of a dog not used in the experimentation.

In addition to the examination of the organs of dogs an attempt was made to detect aluminum in the bone substance. In blank experiments in which aluminum was initially added in quantities of 1 to 20 mg to a certain quantity of bone ash (19)

(corresponding to 20-100 g bovine bone freed of meat and cartilage remnants, defatted and dried), these quantities could not be satisfactorily determined in a single case owing to the invariably excessive calcium content. Attempts to remove the main quantity of calcium as sulfate beforehand in a weakly acid solution or to separate the aluminum as aluminate were not successful.

In bone samples of animals from long-term experiments (with only 10 g initial material) it was nevertheless possible with the method of determination described (but in view of the large quantity of calcium with different quantities of reagents: 5.4 cc hydrochloric acid and 75 cc sodium thiosulfate solution) to determine added quantities of a few milligrams of aluminum approximately by repeated reprecipitation. Without addition, values were obtained which, calculated for aluminum, gave 0.1 to 0.2 mg. This result leads to the conclusion that scarcely any aluminum is deposited in the bones.

Analysis of human urine for aluminum in the experiments with 70 days of daily ingestion (by Dr. Kärber) of aluminum hydroxide suspension gave the following in 1000 cc of urine collected in three days:

Before beginning of experiment.....0.7 mg Al (20)

3 days after beginning of experiment.....0.67 mg Al

At end of experiment.....0.42 mg Al.

2. Excretion of aluminum after administration of single dose of aluminum hydroxide

a) Experiments on dog (20 kg)

1 Kot vom	2 Asche g	3 Al-Gehalt als Al_2O_3 mg*
18. I. (vor Al-Zufuhr) (137 g)	8,26	35,2

4 * Der Al-Gehalt berechnet sich durch Multiplikation mit 0,529.

Key: 1. Stools from: Jan. 18 (before administration of Al). 2. Ash. 3. Al content as Al_2O_3. 4.* Aluminum content is calculated by multiplication by 0.529.

The dog produced no further stools until Jan. 22. On the morning of Jan. 21 he received 100 cc suspension containing 990 mg Al_2O_3 in his food which consisted of 80 g dried meat, 80 g dog biscuits and water, and the entire consumption of which was carefully supervised.

1 Kot vom	2 Asche g	3 Al-Gehalt als Al_2O_3 mg
22.--23. I. (161 g)	9,84	946,0
24. I. kein Kot 4.	—	—
25. I. (140 g) . .	10,59	46,6
26. I. kein Kot .	—	—
27. I. (134 g) . .	8,68	42,1
28. I. (69 g) . . .	3,40	13,5

Key: 1. Stools dated: 2. Ash. 3. Al content as Al_2O_3. 4. No stools.

Aluminum could be detected in the daily quantity of ingested dog biscuits corresponding to 5.4 mg Al_2O_3 and in the daily quantity of ingested dried meat corresponding to 4.3 mg Al_2O_3. In the 24-hour urine of Jan. 21 (450 cc) 0.13 mg aluminum was detected.

Key: 1. Urine from. 2. Stools from. 3. Ash. 4. Al content as Al_2O_3. 5. (before Al ingestion). 6. *The experiment unfortunately had to be broken off due to external circumstances and excretion could therefore not be followed further.

2. On March 31, 1930 the author, after prior urination, ingested 100 cc aluminum hydroxide suspension containing 1020 mg Al_2O_3.

Harn vom	Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
31. III. 250 ccm	—	—	0,17
31. III. 600 „	—	—	0,38
	1. IV.	3,20	423,8
	2. IV.	3,30	425,8
		1,23	99,4
	3. IV.	1,30	51,3
	4. IV.	2,53	2,5

Discussion

In the long-term experiment on dogs fed with aluminum hydroxide no change was observed in the animals' state of health during the period of ingestion, nor were any abnormal findings detected in the organs of the animals in the autopsy (G. Kärber, M.D.). In the dogs receiving aluminum chloride, in agreement with earlier experiments with aluminum salts, there was a sometimes considerable loss of appetite, in one case with persistent loss of weight connected therewith, as well as a slight irritation (autopsy) of the stomach mucosa. The macroscopic and microscopic examination of the organs showed

1. On Feb. 5 the same dog once again received a suspension containing 960 mg Al_2O_3 in his food and was fed until Fb. 9 with raw meat and cooked rice.

1 Kot vom	2 Asche g	Al-Gehalt als Al_2O_3 3 mg
4 5. II. (vor Al-Zufuhr) (20 g)	1,25	9,8
6. II. (25 g)	2,76	857,4
7. II. (30 g)	2,14	47,1
8. II. (34 g)	1,76	27,8

Key: 1. Stools from. 2. Ash. 3. Al content as Al_2O_3.

Thereafter the dog was again fed with dog biscuits and dried meat.

1 Kot vom	2 Asche g	Al-Gehalt als Al_2O_3 3 mg
10. II. (50 g) . .	6,54	33,5
11. II. (45 g) . .	4,19	21,2

Key: 1. Stools from. 2. Ash. 3. Al content in Al_2O_3.

b) Human experiments

On March 6, 1930 the author, after prior urination, ingested 100 cc of an aluminum hydroxide suspension containing 1015 mg Al_2O_3.

1 Harn vom		2 Kot vom	3 Asche g	4 Al-Gehalt als Al_2O_3 mg
6. III.	880 ccm	—	—	0,29
7. III.		—	—	
		5 6. III. (vor der Al-Zufuhr	1,46	4,3
		7. III.	3,93	442,3
		8. III.	3,07	457,7 *

6 * Der Versuch mußte äußerer Umstände wegen leider abgebrochen werden; daher konnte die Ausscheidung nicht weiter verfolgt werden.

no pathological alterations.

No increase in the aluminum content of the organs etc. (Tables I and II) was observed compared to normal values (Table III).

The aluminum quantities detectable in the urine of the experimental dogs did not exceed the traces of aluminum detected in the normal dog urine.

A 70-day ingestion of considerable quantities of aluminum hydroxide did not result in any increased excretion of aluminum in human urine and only at the end of the experiment did it give rise to slight, rapidly subsiding stomach distress.

Even though it cannot be concluded even on the basis of analytical experiments (administration of single doses of aluminum hydroxide) that there is no absorption of aluminum, since under the given conditions a mechanical retention of small quantities of aluminum in the folds of the intestinal mucosa had to be expected for several days and therewith erroneous quantities excreted in the stools (21), the absence of increased aluminum excretion in the urine (with long-term ingestion of Al-hydroxide and Al-chloride), and the absence of any increase in the aluminum content of the organs of animals fed with aluminum compounds suggests that the ingested aluminum does not change the physiological conditions of the passage of traces of aluminum from the gastrointestinal tract into the organism and the urine. This is in accord with the results

of the investigations of G. Kärber (22), according to which the permeability of animal membranes to aluminum salts is extremely slight under normal physiological conditions.

Summary

1. The aluminum content of organs, tissue etc. of dogs was established numerically after the ingestion of aluminum compounds; it ranges on the average around tenths of milligrams per 100 g organic material.

2. In all (7) experimental dogs, after 10 to 15 months of aluminum ingestion, no greater quantities were found in the organs etc. than in the organs etc. of a dog without aluminum ingestion otherwise fed in the usual manner.

3. Slight irritation of the mucosa could only be observed in the dog when readily soluble aluminum salts were administered in large quantities and strong concentrations in the experiments.

4. In human experiments the aluminum administered per os in the form of aluminum hydroxide was almost wholly recovered in the stools. The aluminum content of the urine was not increased by the experimental long-term administration of large quantities of aluminum hydroxide.

5. The result in evaluating conditions in daily life is that aluminum may be regarded as non-absorbable for practical purposes upon ingestion of various kinds of aluminum compounds, even over prolonged periods of time.

Tables.

Tabelle I.

Aluminiumgehalt der Organe usw. der Hunde nach Zufuhr von Aluminiumhydroxydaufschlämmung.
(Die letzte Aluminiumzufuhr erfolgte jedesmal 23 Stunden vor der Sektion.)

1 Organ usw.	2 Hund Nr. 1 Endgewicht: 17,1 kg Versuchsdauer: 10 Monate			Hund Nr. 2 Endgewicht: 16,5 kg Versuchsdauer: 12 Monate			Hund Nr. 3 Endgewicht: 9,5 kg Versuchsdauer: 10 Monate			Hund Nr. 4 Endgewicht: 6,5 kg Versuchsdauer: 13 Monate			Hund Nr. 5 Endgewicht: 8,7 kg Versuchsdauer: 15 Monate		
	3 Gesamtgewicht	Aluminium (Al) in 100 g	4 Aluminium (Al) im Gesamtgewicht	Gesamtgewicht	Aluminium (Al) in 100 g	Aluminium (Al) im Gesamtgewicht	Gesamtgewicht	Aluminium (Al) in 100 g	Aluminium (Al) im Gesamtgewicht	Gesamtgewicht	Aluminium (Al) in 100 g	Aluminium (Al) im Gesamtgewicht	Gesamtgewicht	Aluminium (Al) in 100 g	Aluminium (Al) im Gesamtgewicht
	g	mg	mg	g	mg	mg	g	mg	mg	g	mg	mg	g	mg	mg
4 Magen *	144	0,3	0,44	193	0,2	0,38	84	0,35	0,3	85	0,98 †	0,84 †	90	1,07 †	0,97 †
5 Darm *	—	0,30	—	—	0,33	—	—	0,18	—	—	27,0 †	—	—	0,22	—
6 Blut **	—	0,22	—	—	0,09	—	—	0,18	—	—	0,09	—	—	0,26	—
7 Leber	400	0,39	1,56	375	0,16	0,6	350	0,08	0,28	220	0,13	0,27	207	0,27	0,55
8 Galle	13 ***	—	0,17	15 ***	—	0,13	—	—	—	9 ***	—	0,13	14 ***	—	0,22
9 Niere	95,5	0,13	0,13	70	0,4	0,28	57	0,31	0,18	39	0,56	0,22	57	0,14	0,08
10 Lunge	120	0,22	0,26	126	0,17	0,21	110	0,18	0,2	67	0,19	0,13	77	0,62	0,48
11 Herz	95	0,1	0,09	125	0,15	0,19	98,5	0,24	0,24	72	0,25	0,18	58	0,23	0,13
12 Milz	27,5	0,4	0,11	32	0,22	0,07	15 ***	—	0,09	13 ***	—	0,18	—	—	—
13 Muskel	—	0,3	—	—	0,11	—	—	0,07	—	—	0,09	—	—	0,22	—
14 Gehirn	76	0	0	16	0,12	0,08	—	—	—	—	—	—	71	0,38	0,27

15 * Magen- und Darmwand mit Wasser abgespült.
16 ** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.
17 *** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.
18 † Offenbar verursacht durch kleine, an der Schleimhaut haftende Reste der Aluminiumverbindung von der vorangegangenen Fütterung her, trotz vorheriger Spülung mit Wasser.

I. Aluminum content of organs, etc. of dogs after ingestion of an aluminum hydroxide suspension (the last ingestion of aluminum always took place 23 hours before the autopsy). Key: 1. Organ etc. 2. Dog no. 1. Final weight: 17.1 kg. Duration of experiment: 10 months. 3. Final weight. 4. Stomach*. 5. Intestine*. 6. Blood**. 7. Liver. 8. Bile. 9. Kidney. 10. Lungs. 11. Heart. 12. Spleen. 13. Muscle. 14. Brain. 16.*Stomach and and intestinal wall rinsed off with water. 17. ** Blood was drawn under deep chloroform-ether anesthesia from a canula tied into the carotid. 17. ***In those cases in which the weight of the initial material was only a small fraction of 100 g, we did not recalculate to 100 g. 18. +Apparently caused by small remnants of the aluminum compound adhering to the mucosa from the previous ingestion, despite prior rinsing off with water.

Tabelle II.

Aluminiumgehalt der Organe der Hunde nach Zufuhr von Aluminiumchlorid. (Die letzte Aluminiumzufuhr erfolgte jedesmal 8 Stunden vor der Sektion.)

1 Organ usw.	2 Hund Nr. 6. Endgewicht: 7 kg Versuchsdauer: 8 Monate			Hund Nr. 7 Endgewicht: 18 kg Versuchsdauer: 13 Monate		
		Aluminium (Al)			Aluminium (Al)	
	3 Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht
	g	mg	4 mg	g	mg	mg
5 Magen *	60	0,16	0,11	200	0,93 ††	1,86 ††
6 Darm *	—	0,14	—	—	0,22	—
7 Blut **	—	0,13	—	—	0,27	—
8 Leber	200	0,44	0,88	468	0,22	1,02
9 Galle	12 ***	—	0,09	—	—	—
10 Niere	45	0,18	0,13	53	0,15	0,08
11 Lunge	65	3,7	2,4 †	130	0,62	0,80
12 Herz	60	0,3	0,18	107	0,32	0,35
13 Milz	10 ***	—	0,13	—	—	—
14 Muskel	—	0,4	—	—	0,08	—
15 Gehirn	76	0,16	0,13	85	0,09	0,08

16 * Magen- und Darmwand mit Wasser abgespült.

17 ** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.

18 *** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.

19 † Der verhältnismäßig hohe Aluminiumgehalt dürfte auf den unabhängig vom Versuch in die Lunge des Hundes durch Einatmung gelangten tonerdehaltigen Staub (Erde, Sand usw.) zurückzuführen sein. Vgl. dieselbe Auffassung *C. Oppenheimers* in „Chemie der Zellvorgänge". Gellhorns Lehrb. d. allg. Physiologie 1931, S. 215.

20 †† Offenbar verursacht durch kleine, an der Schleimhaut haftende Reste der Aluminiumverbindung von der vorangegangenen Fütterung her, trotz vorheriger Spülung mit Wasser.

II. Aluminum content of the organs of dogs after ingestion of aluminum chloride. (The last aluminum ingestion always took place 8 hours before the autopsy). Key: 1-18, see Table I. 19. The relatively high aluminum content may have been due to dust containing aluminum oxide (earth, sand, etc.) reaching the lungs of the dog via respiration. See the same view in C. Oppenheimer "Chemistry of Cellular Processes", Gellhørns Lehrbuch d. allg. Physiologie [Gellhorn's Textbook of General Physiology], 1931, p. 245. 20. See 19 in key to Table I.

Tabelle III.

Aluminiumgehalt der Organe eines normalen (nicht mit einer Aluminiumverbindung gefütterten) Hundes.

1 Organ usw.	2 Hund Nr. 8 Gewicht: 12,5 kg		
	3 Gesamtgewicht g	Aluminium (Al)	
		in 100 g mg	im Gesamtgewicht 4a mg
5 Magen *	115	0,17	0,19
6 Darm *	—	0,35	—
7 Blut **	—	0,20	—
8 Leber	321	0,33	1,06
9 Galle	14 ***	—	0,24
10 Niere	60	0,4	0,23
11 Lunge	110	0,29	0,32
12 Herz	91	0,22	0,20
13 Milz	14 ***	—	0,26
14 Muskel	—	0,25	—
15 Gehirn	72	0	0

16 * Magen- und Darmwand mit Wasser abgespült.

17 ** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.

18 *** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.

III. Aluminum content of the organs of a normal dog not fed with aluminum compounds. Key: Same as Table I.

Bibliography

See references in footnotes.

Footnotes other than References

9. Whether the extremely small aluminum content of the organs examined in normal animals given by Steudel (rats: 0.004 to 0.006 mg%), which is several times lower than the data of other authors and the quantities established here, represents the aluminum content present in the organs is not known. When using the colorimetric method of determination employed by him for the experiments described here with added aluminum, satisfactory results were never obtained; as a rule the values were significantly too low.

14. E. Rost gives a comprehensive review of the Al content of foods, human and animal organs etc. in the Hanbuch d. Lebensmittelchem. [Handbook of food chemistry], 1, 1069 and 1073,1933.

16. The aluminum sulfate quantity used as initial material was measured so that 100 cc of the suspension contained roughly 1000 mg aluminum oxide (Al_2O_3). The analytical determination generally gave somewhat lower and somewhat higher values than 1000 mg Al_2O_3 (950 to 1050 mg) per 100 cc.

17. In the case of larger ash quantities (stools) only part of the ash solution was used.

18. Caught in the metabolic cage.

19. The defatted bones were first ashed in a platinum cup in the muffle furnace.

20. According to a recent finding of the National Health Office on the question of the use of aluminum-containing baking powder based on a report from the director of the Medical University Clinic in Kiel, Prof. Schittenhelm, M.D., 0.43 to 1.18 mg Al was detected in the normal human 24-hour urine in their investigations.

21. It should be borne in mind that in dog experiments, as has been observed, there are appreciable quantities of aluminum in the normal stools or in the ingested food, as a result of which it was not possible under present conditions to make an accurate determination of the excretion of the aluminum administered experimentally in the stools.

Biochem Z 265: 169–180 (1933)

Zur Frage der Resorption von Aluminiumverbindungen im Organismus, mit Berücksichtigung des normalen Aluminiumgehalts tierischer Gewebe.

Von

J. Wührer.

(Aus dem pharmakologischen Laboratorium des Reichsgesundheitsamtes.)

(Eingegangen am 29. Juli 1933.)

Nachdem vom gesundheitlichen Standpunkt aus die Frage, ob dauernde Aufnahme kleiner Mengen von Aluminiumverbindungen mit den Speisen gesundheitliche Schädigungen irgendwelcher Art verursachen könne, schon vor geraumer Zeit einer vielseitigen wissenschaftlichen Prüfung unterzogen worden war und auf Grund ihrer Ergebnisse verneint werden konnte[1], ist diese Frage, veranlaßt durch die in der Öffentlichkeit in den letzten Jahren verbreiteten Behauptungen über Gesundheitsschädigungen durch den Gebrauch von Eß-, Trink- und Kochgeschirr aus Aluminium, erneut Gegenstand experimenteller Untersuchungen[2] geworden.

Wesentlich bei der Beurteilung der Frage der pharmakologischen Wirkung von Metallverbindungen im tierischen Organismus ist die Feststellung, in welchem Umfang das Metall vom Magendarmkanal aus

---

[1] *Ohlmüller* u. *Heise*, Arb. a. d. Kaiserl. Ges.-Amt. 8, 377, 1893; *Plagge* u. *Lebbin*, Veröffentl. a. d. Geb. d. Militärsanitätswesens 3, 1893, Berlin; *Lunge* u. *Schmid*, Zeitschr. f. angew. Chem. 7, 1892; *H. Chittenden*, *E. Taylor* u. *H. Long*, Bull. U. S. Depart. of Agriculture Nr. 103, 1914; The Lancet, Some Kitchen Experiments with Aluminium 1, 54, 1913.

[2] *C. Massatsch* u. *H. Steudel*, diese Zeitschr. **220**, 239, 1930; *Steudel*, ebenda **253**, 387, 1932; *C. Massatsch*, Hauszeitschr. d. Vereinigt. Aluminium-Werke usw. Heft 3, S. 75, 1929; die Tierversuche sind gemeinsam mit *H. Steudel* ausgeführt; *K. B. Lehmann*, Arch f. Hyg. **102**, 349; **106**, 336, 1931; *v. Fellenberg*, Mitteil. a. d. Geb. d. Lebensmitteluntersuch. u. Hyg., Bern, **19**, 137, 1928.

resorbiert wird, welchen Verlauf die Ausscheidung des Metalls nimmt und ob das Metall in Organen oder Geweben gespeichert wird. Hinsichtlich des Aluminiums liegen hierüber erst aus letzter Zeit einige umfassende Untersuchungen vor.

Myers und Mitarbeiter[1], sowie *Peterman* und *Underhill*[2] fanden, daß die Zufuhr verhältnismäßig großer Aluminiummengen per os praktisch keinen Einfluß auf den normalen Aluminiumgehalt der Organe und Gewebe von Hunden und Ratten hatte. In normalen tierischen und menschlichen Organen und Geweben wurde Aluminium in geringen Mengen festgestellt, die sich z. B. bei der Leber von 0,15 bis 0,66 mg, beim Blute von 0,06 bis 0,36 mg je 100 g frisches organisches Material bewegten. Auch bei langandauernder Zufuhr von Aluminiumverbindungen in Form von aluminiumhaltigem backpulverhaltigen Biskuits an die Tiere konnte eine Speicherung von Aluminium in den Organen usw. nicht festgestellt werden. Damit konnten die Schlußfolgerungen früherer Untersuchungen von *Steudel*[3] *Kahn*[4] und *Balls*[5], wonach beträchtliche Mengen von Aluminium aufgestapelt werden sollten, als überholt betrachtet werden.

K. Mackenzie[6] hat ferner an umfangreichem Tiermaterial (Ratten und Schweinen) festgestellt, daß die Ausscheidung des per os einverleibten Aluminiums ausschließlich auf den Magendarmkanal beschränkt bleibt und eine nachweisbare Resorption nicht eintritt. *Steudel* (l. c.) nimmt an, daß das in den Verdauungskanal gelangte Aluminiumsalz entweder gar nicht resorbiert wird, oder aber daß es, ähnlich wie das Eisen, nach vorübergehender Resorption in den oberen Dünndarmpartien durch den Dickdarm restlos wieder ausgeschieden wird[7].

Das Vorkommen von Aluminium in normalen tierischen Organen und Geweben haben neben den erwähnten Autoren in neuerer Zeit *Kahlenberg* und *Closs*[8], *Underhill*, *Peterman*, *Gross* und *Krause*[9], *Mas-*

[1] *V. C. Myers* u. *J. W. Mull*, J. of biol. Chem. **78**, 605, 1928; *V. C. Myers* u. *D. B. Morrison*, ebenda **78**, 615, 1928.

[2] *F. P. Underhill* u. *F. J. Peterman*, Amer. J. of Physiol. **90**, 39, 50, 71, 1929.

[3] *M. Steel*, Biochem. Bull. **5**, 173, 1916, zit. nach *Myers* u. *Morrison*, J. of biol. Chem. **78**, 624, 1928.

[4] *M. Kahn*, Biochem. Bull. **1**, 235, 1911/12, zit. nach *Myers* u. *Morrison*, J. of biol. Chem. **78**, 624, 1928.

[5] *A. K. Balls*, Biochem. Bull. **5**, 195, 1916, zit. nach *Myers* u. *Morrison*, J. of biol. Chem. **78**, 624, 1928.

[6] *K. Mackenzie*, Biochem. J. **24**, 1433, 1930; **25**, 287, 1931.

[7] Ob der von *Steudel* angegebene äußerst kleine Aluminiumgehalt der untersuchten Organe normaler Tiere (Ratten: 0,004 bis 0,006 mg-%), der gegenüber den Angaben anderer Autoren und auch den hier festgestellten Mengen um ein Vielfaches geringer ist, den in den Organen vorhandenen Aluminiumgehalt darstellt, muß dahingestellt bleiben. Bei der Benutzung des von ihm angewendeten kolorimetrischen Bestimmungsverfahrens zu den hier angestellten Versuchen mit zugesetztem Aluminium wurden in keinem Falle befriedigende, meist bedeutend zu niedrige Werte erhalten.

[8] *L. Kahlenberg* u. *J. O. Closs*, J. of biol. Chem. **83**, 261, 1929.

[9] *Underhill, Peterman, Gross* u. *Krause*, Amer. J. of Physiol. **90**, 72, 1929.

satsch (l. c.), *K. B. Lehmann*[10] und *S. J. Lewis*[11] festgestellt[12]. Die Ergebnisse der Untersuchungen von *Gonnermann*[13] über den Gehalt menschlicher Organe an Aluminium — es wurden dort Werte von 0,2 bis 3,4 % der Trockensubstanz gefunden — sind angesichts der analytisch nicht einwandfreien Bestimmungsmethode unmöglich als richtig anzusehen und nach allen vorliegenden Untersuchungen und eigenen Erfahrungen überholt.

In früher angestellten, bisher unveröffentlichten Untersuchungen des Laboratoriums über die pharmakologischen Wirkungen des Aluminiums trat bei Hunden eine teilweise Herabminderung der Freßlust ein, wenn sauer reagierende Aluminiumsalze im Futter während mehrerer Wochen täglich verabreicht wurden. (Kalialaun: 3,2 bis 6,4 g, Aluminiumsulfat: 4,5 g und Aluminiumacetatlösung: entsprechend 0,125 bis 0,25 g Al_2O_3 je Tier). Im Harn der Tiere konnte Aluminium nicht, in den Darmentleerungen die Gesamtmenge des zugeführten Aluminiums wieder festgestellt werden. Hieraus wurde geschlossen, daß auch bei längerer Zufuhr großer Aluminiumsalzmengen an Hunde sich keine schädliche resorptive Wirkung zeigt, und daß die beobachtete Abnahme der Freßlust auf die örtlich adstringierende, zum Teil ätzende Wirkung dieser großen Mengen stark hydrolysierter Aluminiumsalze zurückzuführen ist.

Die Einverleibung großer Mengen löslicher, Schleimhautreizungen hervorrufender Aluminiumsalze kann hinsichtlich der pharmakologischen Beurteilung nicht mit der Aufnahme kleiner, mit der Nahrung zugeführter Aluminiumverbindungen verglichen werden. Es war daher zu prüfen, ob bei sehr lange andauernder Zufuhr kleiner Mengen eines leicht löslichen Aluminiumsalzes mit dem täglichen Futter einerseits und bei ebensolcher Verabreichung großer Mengen einer Aluminiumverbindung, die keine wesentliche Reizwirkung auf die Schleimhaut ausübt, andererseits eine Resorption und Speicherung von Aluminium in Organen und Geweben sich feststellen läßt. Zu den nachfolgend beschriebenen Versuchen wurde einerseits Aluminiumchlorid ($AlCl_3 + 6\,H_2O$) in Gelatinekapseln und andererseits frisch gefälltes Aluminiumhydroxyd (das beträchtliche Löslichkeit in schwachsauren Flüssigkeiten zeigt) zur Fütterung verwendet.

Um ein möglichst in gleichmäßiger Form zu verfütterndes Aluminiumhydroxyd zu erhalten, wurde eine Lösung von kristallisiertem Aluminiumsulfat (60 g) in der Siedehitze mit einem geringen Überschuß von Ammoniak gefällt, sofort noch heiß abgesaugt und heiß ausgewaschen. Der Aluminiumhydroxydbrei wurde mit Wasser versetzt, zum Sieden erhitzt, am Rührwerk etwa 2 Stunden gerührt und durch ein feinmaschiges Sieb gegossen. Die emulsionsartige Aufschlämmung wurde dann in einem Meßkolben auf

[10] *K. B. Lehmann*, Arch. f. Hyg. **106**, 309, 1931.

[11] *S. J. Lewis*, Biochem. J. **25**, Teil 3, 2162, 1931.

[12] *E. Rost* gibt eine umfassende Übersicht über den Al-Gehalt von Lebensmitteln, menschlichen und tierischen Organen usw. in dem Handb. d. Lebensmittelchem. **1**, 1069 u. 1073, 1933.

[13] *M. Gonnermann* (aus dem Institut *Koberts*), diese Zeitschr. **88**, 401, 1918.

1000 ccm mit Wasser aufgefüllt. 100 ccm der Aufschlämmung, deren Gehalt an Al_2O_3 jeweils bestimmt wurde, wurden für die Fütterungszwecke mit dem täglichen Futter (gekochter Reis, getrocknetes oder frisches, gekochtes Fleisch) vermengt und den Hunden verabreicht. Das mit der Aufschlämmung gemischte Futter wurde im allgemeinen restlos von den Tieren aufgenommen.

Auf diese Weise wurden 5 Hunde monatelang gleichmäßig täglich anfangs mit kleineren Mengen (150, 300, 600 mg, steigend), in der zweiten Hälfte der Fütterungszeit mit durchschnittlich 1000 mg Aluminiumoxyd[1] enthaltenden Aufschlämmungen gefuttert (vgl. Tabelle I).

Die Hunde (Nr. 1 bis 5) erhielten im ganzen 190, 210, 225, 292 bzw. 337 g Aluminiumoxyd auf die angegebene Weise zugeführt. Das Aluminiumchlorid ($AlCl_3 \cdot 6\,H_2O$) wurde in fester Form in Gelatinekapseln an zwei Hunde, und zwar täglich 300 mg Aluminiumchlorid entsprechend 33 mg Aluminium, morgens unmittelbar vor der üblichen Nahrungsaufnahme eingegeben (vgl. Tabelle II).

Sodann wurden in Einzelversuchen am Hund und auch am Menschen nach Verabreichung einer Einzeldosis von etwa 1000 mg Al_2O_3 enthaltender Aluminiumhydroxydaufschlämmung Harn und Kot teils nach Verlauf mehrerer Stunden, teils nach Tagen auf Aluminium untersucht. Weiterhin nahmen zwei Versuchspersonen (Verfasser und Dr. *Körber*) während 17 Tagen täglich je etwa 1000 mg Al_2O_3 enthaltende Aufschlämmung ein. Die eine der beiden Versuchspersonen (Dr. *Körber*) setzte den Versuch bei einer täglichen Einnahme von etwa 500 mg Al_2O_3 enthaltenden Aufschlämmung bis zur Dauer von 70 Tagen fort.

4. Bestimmung des Aluminiums im organischen Material.

Von den den getöteten Tieren entnommenen Organen wurden jeweils 100 g — wenn das Einzelorgan geringer im Gewicht war, das ganze Organ — zur Analyse verwendet. Um den Analysengang möglichst abzukürzen und Fehler durch Zusätze größerer Mengen von Reagenzien (Säuren) bei der Mineralisierung nach Möglichkeit auszuschalten, wurde von einer Zerstörung der Organe auf nassem Wege abgesehen und, da ein Verlust durch Flüchtiggehen von Aluminium beim Veraschen und vorsichtigem Glühen nicht zu befürchten ist, die Organe nach vorheriger Trocknung in Quarzschalen über freier Flamme unter tropfenweiser Zugabe kleiner Mengen konz. Salpetersäure (3 bis 5 ccm) verascht. Schwer verbrennbare Kohle wurde mit Wasser ausgelaugt, für sich verbrannt und die Auslaugeflüssigkeit der Asche wieder zugesetzt. Ebenso wurde auch mit dem Kot verfahren. Der Harn wurde eingedampft, die organische Substanz mit rauchender Salpetersäure zerstört und zuletzt in der Quarzschale verascht. Die Asche wurde in 10 bis 15 ccm 25%iger Salzsäure in der Platin- oder Quarzschale unter Erwärmen gelöst und, wenn nötig, von dem meist sehr geringen, aus Kieselsäure bestehenden unlöslichen Rückstand abfiltriert. In den Fällen, in denen geringe Mengen von Kupfer (insbesondere Leber) zu erwarten waren, wurde die salzsaure

[1] Die als Ausgangsmaterial verwendete Aluminiumsulfatmenge war so bemessen, daß 100 ccm der Aufschlämmung etwa 1000 mg Aluminiumoxyd (Al_2O_3) enthielten. Die analytische Gehaltsbestimmung ergab meist etwas kleinere oder etwas größere Werte als 1000 mg Al_2O_3 (950 bis 1050 mg) pro 100 ccm.

Lösung entsprechend verdünnt, mit Schwefelwasserstoff gefällt, mehrere Stunden stehengelassen, filtriert und auf etwa 50 ccm eingedampft[1]. Die Lösung der Asche wurde mit Ammoniak bis zur noch eben sauren Reaktion (Phenolphthalein als Indikator) neutralisiert, hierauf 1,8 ccm Salzsäure (sp. Gew. 1,19) zugesetzt, auf etwa 400 ccm verdünnt und nacheinander 10 ccm 10%ige Dinatriumphosphatlösung, 25 ccm 20%ige Natriumthiosulfatlösung und 7,5 ccm 30%ige Essigsäure hinzugefügt.

Trat beim Zusatz von Natriumphosphat eine nicht wieder verschwindende Fällung ein, wie z. B. bei verhältnismäßig hohem Eisengehalt (Blut, Leber), so wurden weitere 1,8 ccm Salzsäure und 25 ccm Natriumthiosulfatlösung zugesetzt. Die sich durch beginnende Schwefelausscheidung trübende Lösung wurde ½ Stunde zum Kochen unter Ersatz des verdampften Wassers erhitzt und nach dem Absetzenlassen des aus Schwefel und Aluminiumphosphat bestehenden Niederschlags abfiltriert, heiß ausgewaschen und der Niederschlag samt Filter im Platintiegel getrocknet, verascht, geglüht und gewogen. Hiernach gelingt es, auch bei Gegenwart von Eisen und Calcium das Aluminium als Phosphat zu fällen, ohne daß Eisen und Calcium mitgefällt werden.

In Blindversuchen mit Aluminiumsulfatlösung [1,23 g $Al_2(SO_4)_3 \cdot 18\,H_2O$ in 1 Liter Wasser gelöst] ergaben sich folgende Werte. Zu 20 ccm destilliertem Wasser wurden

Zugesetzt Al in mg . .	10,0	5,0	2,0	1,5	1,0	0,5	0,4	0,3	0,2	0,1
Gefunden „ „ „ . . .	9,9	4,95	1,9	1,5	0,99	0,5	0,44	0,27	0,22	0,15

Ohne Aluminiumzusatz ergab sich ein Durchschnittsblindwert von 0,3 mg, der, als Aluminium berechnet, 0,06 mg Al ergibt und von sämtlichen ermittelten Werten in Abzug gebracht wurde.

Zerkleinerten Organen (je 100 g Muskel, Leber, Darm) wurde

Zugesetzt Al in mg . .	5,0	3,0	2,0	2,0	1,5	1,0	0,6	0,35	0,2
Gefunden „ „ „ . . .	4,95	3,0	2,04	2,1	1,37	0,85	0,69	0,3	0,2 *

* Die ohne Aluminiumzusatz erhaltenen Werte (s. Tabelle) sind in Abzug gebracht.

Hiernach ermöglicht die Methode auch die Erfassung sehr kleiner hier in Frage kommender Mengen von Aluminium in organischem Material mit befriedigender Genauigkeit und einer Fehlergrenze von etwa ± 20%. Die von *Underhill* und *Peterman* (l. c.) angegebene kolorimetrische Methode lieferte in Kontrollversuchen mit den gewichtsanalytischen Werten gut übereinstimmende Zahlen.

II. Versuchsergebnisse.

1. Dauerversuche.

Die in den Organen usw. ermittelten Aluminiumwerte sind in nachstehenden Tabellen wiedergegeben:

[1] Bei größeren Aschenmengen (Kot) wurde nur ein Teil der Aschenlösung verwendet.

Tabelle I.

Aluminiumgehalt der Organe usw. der Hunde nach Zufuhr von Aluminiumhydroxydaufschlämmung.
(Die letzte Aluminiumzufuhr erfolgte jedesmal 23 Stunden vor der Sektion.)

Organ usw.	Hund Nr. 1 Endgewicht: 17,1 kg Versuchsdauer: 10 Monate			Hund Nr. 2 Endgewicht: 16,5 kg Versuchsdauer: 12 Monate			Hund Nr. 3 Endgewicht: 9,5 kg Versuchsdauer: 10 Monate			Hund Nr. 4 Endgewicht: 6,5 kg Versuchsdauer: 14 Monate			Hund Nr. 5 Endgewicht: 4,7 kg Versuchsdauer: 15 Monate		
	Aluminium (Al)			Aluminium (Al)			Aluminium (Al)			Aluminium (Al)			Aluminium (Al)		
	Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht
	g	mg	mg	g	mg	mg	g	mg	mg	g	mg	mg	g	mg	mg
Magen *	144	0,3	0,44	193	0,2	0,38	84	0,35	0,3	85	0,98 †	0,84 †	90	1,07 †	0,97 †
Darm *	—	0,39	—	—	0,33	—	—	0,18	—	—	27,0 †	—	—	0,22	—
Blut **	—	0,22	—	—	0,09	—	—	0,18	—	—	0,09	—	—	0,26	—
Leber	400	0,39	1,56	375	0,16	0,6	350	0,08	0,28	220	0,13	0,27	207	0,27	0,55
Galle	13 ***	—	0,17	15 ***	—	0,13	—	—	—	9 ***	—	0,13	14 ***	—	0,22
Niere	95,5	0,13	0,13	70	0,4	0,28	57	0,31	0,18	39	0,56	0,22	57	0,14	0,08
Lunge	120	0,22	0,26	126	0,17	0,21	110	0,18	0,2	67	0,19	0,13	77	0,62	0,48
Herz	93	0,1	0,09	125	0,15	0,19	98,5	0,24	0,24	72	0,25	0,18	53	0,23	0,13
Milz	27,5	0,4	0,11	32	0,22	0,07	15 ***	—	0,09	13 ***	—	0,18	—	—	—
Muskel	—	0,3	—	—	0,11	—	—	0,07	—	—	0,09	—	—	0,22	—
Gehirn	76	0	0	16	0,12	0,08	—	—	—	—	—	—	71	0,38	0,27

* Magen- und Darmwand mit Wasser abgespült.
** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.
*** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.
† Offenbar verursacht durch kleine, an der Schleimhaut haftende Reste der Aluminiumverbindung von der vorangegangenen Fütterung her, trotz vorheriger Spülung mit Wasser.

Tabelle II.

Aluminiumgehalt der Organe der Hunde nach Zufuhr von Aluminiumchlorid. (Die letzte Aluminiumzufuhr erfolgte jedesmal 8 Stunden vor der Sektion.)

Organ usw.	Hund Nr. 6. Endgewicht: 7 kg Versuchsdauer: 8 Monate			Hund Nr. 7 Endgewicht: 18 kg Versuchsdauer: 13 Monate		
		Aluminium (Al)			Aluminium (Al)	
	Gesamtgewicht	in 100 g	im Gesamtgewicht	Gesamtgewicht	in 100 g	im Gesamtgewicht
	g	mg	mg	g	mg	mg
Magen *	60	0,16	0,11	200	0,93 ††	1,86 ††
Darm *	—	0,14	—	—	0,22	—
Blut **	—	0,13	—	—	0,27	—
Leber	200	0,44	0,88	468	0,22	1,02
Galle	12 ***	—	0,09	—	—	—
Niere	45	0,18	0,13	53	0,15	0,08
Lunge	65	3,7	2,4 †	130	0,62	0,80
Herz	60	0,3	0,18	107	0,32	0,35
Milz	10 ***	—	0,13	—	—	—
Muskel	—	0,4	—	—	0,08	—
Gehirn	76	0,16	0,13	85	0,09	0,08

* Magen- und Darmwand mit Wasser abgespült.
** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.
*** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.
† Der verhältnismäßig hohe Aluminiumgehalt dürfte auf den unabhängig vom Versuch in die Lunge des Hundes durch Einatmung gelangten tonerdehaltigen Staub (Erde, Sand usw.) zurückzuführen sein. Vgl. dieselbe Auffassung *C. Oppenheimers* in „Chemie der Zellvorgänge", Gellhorns Lehrb. d. allg. Physiologie 1931, S. 245.
†† Offenbar verursacht durch kleine, an der Schleimhaut haftende Reste der Aluminiumverbindung von der vorangegangenen Fütterung her, trotz vorheriger Spülung mit Wasser.

Im Tagesharn (24 Stunden)[1] der im Dauerversuch mit Aluminiumhydroxyd und der mit Aluminiumchlorid gefütterten Tiere konnte Aluminium nicht oder nur in Spuren (bis 0,15 mg Al) nachgewiesen werden, ebenso wie auch im Tageshorn eines nicht zu Versuchen verwendeten Hundes.

Neben der Untersuchung der Organe der Hunde wurde versucht, Aluminium auch in der Knochensubstanz nachzuweisen. In Blindversuchen, bei denen anfangs zu einer bestimmten Knochenascheumenge[2] (entsprechend 20 bis 100 g, von Fleisch und Knorpelresten befreiter, entfetteter und getrockneter Rinderknochen) Aluminium in Mengen von 1 bis 20 mg zugesetzt wurde, konnten diese Mengen infolge des stets störenden übergroßen Calciumgehalts in keinem Falle quantitativ befriedigend wieder bestimmt werden. Versuche, die Hauptmenge des Calciums als Sulfat in schwach saurer Lösung vorher zu entfernen oder das Aluminium als Aluminat abzutrennen, führten nicht zum Ziele.

[1] Im Stoffwechselkäfig aufgefangen.
[2] Die entfetteten Knochen wurden zuerst in der Platinschale im Muffelofen verascht.

Tabelle III.

Aluminiumgehalt der Organe eines normalen (nicht mit einer Aluminiumverbindung gefütterten) Hundes.

Organ usw.	Hund Nr. 8 Gewicht: 12,5 kg Gesamtgewicht g	Aluminium (Al) in 100 g mg	Aluminium (Al) im Gesamtgewicht mg
Magen*	115	0,17	0,19
Darm*	—	0,35	—
Blut**	—	0,20	—
Leber	321	0,33	1,06
Galle	14***	—	0,24
Niere	60	0,4	0,23
Lunge	110	0,29	0,32
Herz	91	0,22	0,20
Milz	14***	—	0,26
Muskel	—	0,25	—
Gehirn	72	0	0

* Magen- und Darmwand mit Wasser abgespült.

** Blut wurde in tiefer Chloroform-Äthernarkose aus einer in die Carotis eingebundenen Kanüle entnommen.

*** In den Fällen, in denen das Gewicht des Ausgangsmaterials nur einen kleinen Bruchteil von 100 g betrug, wurde die Umrechnung auf 100 g nicht vorgenommen.

In Knochenproben der Tiere des Dauerversuchs (bei nur 10 g Ausgangsmaterial) konnten mit der beschriebenen Bestimmungsmethode (der großen Calciummenge entsprechend jedoch abgeänderte Reagenziënmengen: 5,4 ccm Salzsäure und 75 ccm Natriumthiosulfatlösung) durch mehrmaliges Umfällen zugesetzte Mengen von einigen Milligramm Aluminium annähernd bestimmt werden. Ohne Zusatz ergaben sich hierbei Werte, die, auf Aluminium berechnet, 0,1 bis 0,2 mg ergaben. Dieses Ergebnis läßt wohl darauf schließen, daß in den Knochen eine Ablagerung von Aluminium kaum stattfindet.

Die Untersuchung des Menschenharns auf Aluminium im Versuch der 70tägigen täglichen Einnahme (Dr. *Kärber*) von Al-Hydroxydaufschlämmung ergab in 1000 ccm des dreitägigen Sammelharns

vor Beginn des Versuchs	0,7 mg Al[1]
3 Tage nach Beginn des Versuchs	0,67 „ Al
am Ende des Versuchs	0,42 „ Al

[1] Nach einem dem Reichsgesundheitsamt vorliegenden neueren Gutachten über die Frage der Verwendung aluminiumhaltiger Backpulver von dem Direktor der Mediz. Universitätsklinik in Kiel, Prof. Dr. *Schittenhelm*, wurden bei den dortigen Untersuchungen im normalen menschlichen Tagesharn 0,43 bis 1,18 mg Al festgestellt.

2. Ausscheidung von Aluminium nach Verabreichung von Einzeldosen von Al-Hydroxyd.

a) Versuche am Hund (20 kg).

Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg*
18. I. (vor Al-Zufuhr) (137 g)	8,26	35,2

* Der Al-Gehalt berechnet sich durch Multiplikation mit 0,529.

Der Hund gab keinen Kot mehr bis zum 22. Januar. Er erhielt am 21. Januar morgens 100 ccm Aufschlämmung, enthaltend 990 mg Al_2O_3, in das Futter, welches aus je 80 g getrocknetem Fleisch, 80 g Hundekuchen und Wasser bestand, und dessen restlose Aufnahme genau überwacht wurde.

Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
22.—23. I. (161 g)	9,84	946,0
24. I. kein Kot	—	—
25. I. (140 g)	10,59	46,6
26. I. kein Kot	—	—
27. I. (134 g)	8,68	42,1
28. I. (69 g)	3,40	13,5

In der täglich verfütterten Menge Hundekuchen konnte Aluminium, entsprechend 5,4 mg Al_2O_3, und in der täglich verfütterten Menge getrockneten Fleisches, entsprechend 4,3 mg Al_2O_3, festgestellt werden. Im Tagesharn vom 21. Januar dieses Hundes (450 ccm) wurden 0,13 mg Al gefunden.

Derselbe Hund erhielt wieder am 5. Februar 960 mg Al_2O_3 enthaltende Aufschlämmung ins Futter und wurde bis zum 9. Februar nur mit rohem Fleisch und gekochtem Reis gefüttert.

Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
5. II. (vor Al-Zufuhr) (20 g)	1,25	9,8
6. II. (25 g)	2,76	857,4
7. II. (30 g)	2,14	47,1
8. II. (34 g)	1,76	27,8

Danach wieder Fütterung mit Hundekuchen und getrocknetem Fleisch.

Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
10. II. (50 g)	6,54	33,5
11. II. (45 g)	4,19	21,2

b) Versuche am Menschen.

1. Verfasser nahm (6. März 1930) nach vorheriger Harnblasenentleerung 100 ccm 1015 mg Al_2O_3 enthaltende Al-Hydroxydaufschlämmung ein.

Harn vom		Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
6. III.	880 ccm	—	—	0,29
7. III.		—	—	
		6. III. (vor der Al-Zufuhr	1,46	4,3
		7. III.	3,93	442,3
		8. III.	3,07	457.7 *

* Der Versuch mußte äußerer Umstände wegen leider abgebrochen werden; dabei konnte die Ausscheidung nicht weiter verfolgt werden.

2. Verfasser nahm (31. März 1930) nach vorheriger Harnblasenentleerung 100 ccm 1020 mg Al_2O_3 enthaltende Al-Hydroxydaufschlämmung ein.

Harn vom		Kot vom	Asche g	Al-Gehalt als Al_2O_3 mg
31. III.	250 ccm	—	—	0.17
	600 „	—	—	0,38
		1. IV.	3,20	423.8
		2. IV.	3,30	425,8
			1,23	99,4
		3. IV.	1,30	51,3
		4. IV.	2,53	2,5

Besprechung der Ergebnisse.

Bei den im Dauerversuch mit Aluminiumhydroxyd gefütterten Hunden konnte weder während der Fütterungsperiode eine Veränderung im Gesundheitszustand der Tiere festgestellt werden, noch konnten an den Organen der Tiere bei der Sektion (Dr. med. *G. Kärber*) anormale Befunde beobachtet werden. Bei den mit Aluminiumchlorid gefütterten Hunden zeigten sich in Übereinstimmung mit den früheren Versuchen mit Aluminiumsalzen zeitweise beträchtliche Herabsetzung der Freßlust und in einem Fall auch damit verbundene dauernde Gewichtsabnahme, sowie (bei der Sektion) geringe Reizwirkungen an der Magenschleimhaut. Die makro- und mikroskopische Untersuchung der Organe der Tiere ergab keine pathologischen Veränderungen.

Eine Steigerung des Aluminiumgehalts der Organe usw. (Tabelle I und II) gegenüber den Normalwerten (Tabelle III) ist nicht zu erkennen.

Die im Harn der Versuchshunde feststellbaren Aluminiummengen überstiegen nicht die im normalen Hundeharn sich findenden Spuren von Aluminium.

Eine 70tägige Einnahme beträchtlicher Mengen von Aluminiumhydroxyd hatte auch beim Menschen keine vermehrte Aluminiumausscheidung im Harn zur Folge und verursachte nur am Ende des Versuchs geringfügige, schnell vorübergehende Magenbeschwerden.

Wenn auch aus den Bilanzversuchen (Verabreichung von Einzeldosen von Al-Hydroxyd) nicht geschlossen werden kann, daß eine Resorption von Aluminium nicht eintritt, da bei den gegebenen Bedingungen mit einer mechanischen Zurückhaltung kleiner Mengen des Aluminiums in den Falten der Darmschleimhaut auf mehrere Tage hin, und daher mit Fehlmengen bei der Ausscheidung im Kot zu rechnen war[1], so spricht doch das Fehlen einer vermehrten Aluminiumausscheidung im Harn (bei der Dauerverabreichung von Al-Hydroxyd und Al-chlorid), sowie ein nicht vermehrter Aluminiumgehalt der Organe der mit Aluminiumverbindungen gefütterten Tiere dafür, daß das zugeführte Aluminium die physiologischen Verhältnisse des Übertritts von Spuren Aluminium vom Magendarmkanal aus in den Organismus und in den Harn nicht ändert. Damit in Übereinstimmung stehen die Ergebnisse der Untersuchungen von *G. Kärber*[2], wonach die Durchdringbarkeit tierischer Membranen für Aluminiumsalze unter den normalen physiologischen Bedingungen nur eine äußerst geringe ist.

Zusammenfassung.

1. Es wird der Gehalt der Organe, Gewebe usw. von Hunden an Aluminium bei der Zufuhr von Aluminiumverbindungen zahlenmäßig festgestellt; er bewegt sich durchschnittlich in der Größenordnung von Zehntelmilligrammen je 100 g organisches Material.

2. Bei sämtlichen (7) Versuchshunden wurden nach 10 bis 15monatlicher Aluminiumzufuhr in den Organen usw. keine größeren Aluminiummengen gefunden, als in den Organen usw. eines Hundes ohne Aluminiumzufuhr bei der üblichen Fütterung nachweisbar waren.

[1] Bei den Versuchen am Hund ist zu berücksichtigen, daß im normalen Kot bzw. in der aufgenommenen Nahrung, wie sich gezeigt hat, nicht unerhebliche Mengen von Aluminium enthalten sind, wodurch unter den angegebenen Bedingungen eine genaue Erfassung der Ausscheidung des versuchsweise zugeführten Aluminiums im Kot nicht möglich war.

[2] *G. Kärber*, Arch. f. exper. Pathol. u. Pharm; erscheint demnächst.

3. Geringfügige schleimhautreizende Wirkungen ließen sich bei den Hunden nur dann feststellen, wenn leicht lösliche Aluminiumsalze in größeren Mengen und in nicht unbeträchtlichen Konzentrationen im Versuch verabreicht wurden.

4. Im Versuch am Menschen konnte das per os in Form von Aluminiumhydroxydgel zugeführte Aluminium im Kot fast restlos wiedergefunden werden. Durch eine versuchsweise, lang andauernde Aluminiumhydroxydverabreichung in beträchtlichen Mengen wurde der Aluminiumgehalt des Harns nicht erhöht.

5. Für die Beurteilung der Verhältnisse im täglichen Leben ergibt sich, daß bei der Zufuhr von Aluminiumverbindungen verschiedener Art, selbst bei langandauernder Verabreichung, Aluminium als praktisch nicht resorbierbar zu betrachten ist.